JOURNAL OF AGRICULTURAL RESEARCH
DEPARTMENT OF AGRICULTURE

VOL. VI WASHINGTON, D. C., JULY 31, 1916 No. 18

LIFE CYCLES OF THE BACTERIA

[PRELIMINARY COMMUNICATION]

BY F. LÖHNIS, *Soil Biologist*, and N. R. SMITH, *Scientific Assistant, Bureau of Plant Industry*.[1]

INTRODUCTION

Two years ago the senior author, together with J. Hanzawa (14),[2] published the results of some Azotobacter studies, showing for the first time that the large Azotobacter cells are a special type of growth of a spore-forming bacillus. We said (14, p. 2):

Es steht jetzt fest, dass in der Tat die grossen, sporenfreien Azotobacter-Zellen Wuchsformen eines schlanken, Endosporen bildenden Bacillus sind. Bacillus Azotobacter ist demnach die korrekte Bezeichnung für diese Art.[3]

As we had to discontinue our investigations at that time, we pointed out (14, p. 6) that further research in this direction would be very desirable:

Sicherlich würden weitere Forschungen in dieser Richtung noch manchen für den Systematiker wie für den Physiologen gleich wichtigen Einblick erschliessen.

In the meantime some new papers on Azotobacter have been published. But they merely confirmed once more certain facts concerning the normal growth and the rather general occurrence of Azotobacter in soils. Only one author, Mulvania (15), reports the presence of heat-resisting spores. In the other cases no spores were observed. However, they undoubtedly would have been found by a more thorough search. One of these authors readily admitted this fact in a letter, saying that his statement had been made "on the basis of the ordinary examination always made by soil bacteriologists."

[1] The photomicrographs, as well as the final drawing of the text figure accompanying this paper, have been made by Mr. F. L. Goll, of the Bureau of Plant Industry. Grateful acknowledgment is due him for his very careful work.

[2] Reference is made by number to "Literature cited," p. 701-702.

[3] In accordance with the usage of K. B. Lehmann and most of the other European bacteriologists, we apply the name "Bacillus" to the spore-forming rods. As *Bacillus azotobacter*, like most, probably all, spore-forming rods, has, at least temporarily, peritrichous flagella, its name would also be valid if Migula's system should be preferred. However, we fully agree with Lehmann, that this system is especially unsatisfactory.

As our earlier observations indicated that an extended study would lead to still more interesting results, we have resumed our work. A comparative study of 24 Azotobacter cultures and 18 strains of other bacteria now revealed the fact that those wide morphological differences first observed with Azotobacter are by no means restricted to this one group of bacteria. Similar variations occurred with all cultures tested, and under suitable conditions they will occur with all bacteria generally. The importance of these wide morphological variations, however, is materially increased by the fact that they are connected with no less considerable variations in the physiological qualities of those organisms. Therefore, not only for diagnostic and systematic purposes are these facts of fundamental importance but also for all other lines of research in agricultural and medical bacteriology.

The quite unexpected character of the results obtained seems to justify a preliminary discussion of the facts and problems involved. Of course, at the present time it is neither our intention to furnish all those numerous details which are necessary to obtain a full knowledge of these heretofore practically unknown facts, nor do we want to collect all the widely scattered observations from a voluminous literature which will not only give some interesting support to our new viewpoint but which also, in their turn, will sometimes find their full explanation there. At present we merely wish to inform agricultural and also medical bacteriologists about these newly discovered facts and to ask for their cooperation.

It is beyond question that progress in bacteriology has been severely checked by the widespread inclination to consider as not worth studying or as some uninteresting "involution form" all that sort of bacterial growth which does not fit exactly into the conventional conception of a very simple and constant character of the species. Even modern standard works assert, for instance, that the branched type of *Bacillus radicicola* represents an "involution form" not capable of further propagation. However, nitrogen fixation takes place only when these branched forms develop, which unmistakably proves their full virility; and there is no lack of exact results which show conclusively that suitable conditions always allow a new development from these branched forms.

Undoubtedly a somewhat more scientific study of such "abnormal" forms would long ago have revealed the fact that the life cycles of the bacteria are no less complicated than those of many other micro-organisms. Indeed, numerous items in the bacteriological literature, for instance, show that the formation of gonidia and the budding of bacteria have been observed quite frequently. Yet again the authoritative statement that bacteria multiply exclusively by fission apparently has been sufficient to prevent thorough research in this direction, and the credulous adherence to "standard methods" unfortunately explains only too well why the turning point in the life cycles of the bacteria has been com-

pletely overlooked. In fact, this slime or granulated dirt has been merely an annoying occurrence on the slides of thousands of bacteriologists. Acetic acid and many other remedies have been recommended to insure clean preparates. Of course, beef broth and some other substrates usually give really dirty smears which need some cleaning, but we have been much too radical in this direction. Under certain conditions all bacteria pass over into a "symplastic" stage, appearing under the microscope as either an unstainable or a readily stainable mass without any visible organization, which, if not discarded as dead, later gives birth to new regenerated forms frequently of very characteristic and unusual appearance.

As practically all our new knowledge of the life cycles of the bacteria has been derived from a renewed study of *B. azotobacter*, the behavior of this organism will be described first.

Before we enter into this subject, however, we beg to point out that by discussing the life cycles of the bacteria we do not intend to revive any of those unclear theories concerning bacterial polymorphism or pleomorphism. The development of the bacteria is characterized not by the *irregular* occurrence of more or less *abnormal* forms but by the *regular* occurrence of many different forms and stages of growth connected with each other by *constant relations*.

Unquestionably many so-called species frequently described in the most superficial manner will have to be canceled, because they merely represent fragments of the life cycles of other bacteria. "Good" species, on the other hand, will not only keep their position, but they will receive a much more complete and sharper definition than they now have. Moreover, the discovery of the symplastic stage opens the way to answer by exact experiments the question concerning species or varieties.

THE LIFE CYCLE OF BACILLUS AZOTOBACTER

In text figure 1 is given a schematic sketch of the development of *B. azotobacter* according to our present knowledge. The letters A to M indicate the different types of growths which are separated from each other by broken lines. The single- and double-pointed arrows show the connections between the different forms as they have actually been observed. Each of the four circles contain in each case all those forms which have heretofore been considered by careful investigators as representing sufficient basis for establishing a species. Observers of the more usual, less painstaking class, however, have been only too much inclined to form new species even inside these subcycles. For example, the different types of spore-free and spore-bearing rods, all included in our type F, could easily have induced authors like Migula and Matzushita to create half a dozen "species" of that sort; perhaps this really happened.

With the exception of D and H, all these types have been observed and described in earlier publications on Azotobacter and closely related

spore-forming bacilli (*B. malabarensis*, *B. danicus*, and *B. oxalaticus*). However, figure 1 shows clearly how just this type D, which has hereto-

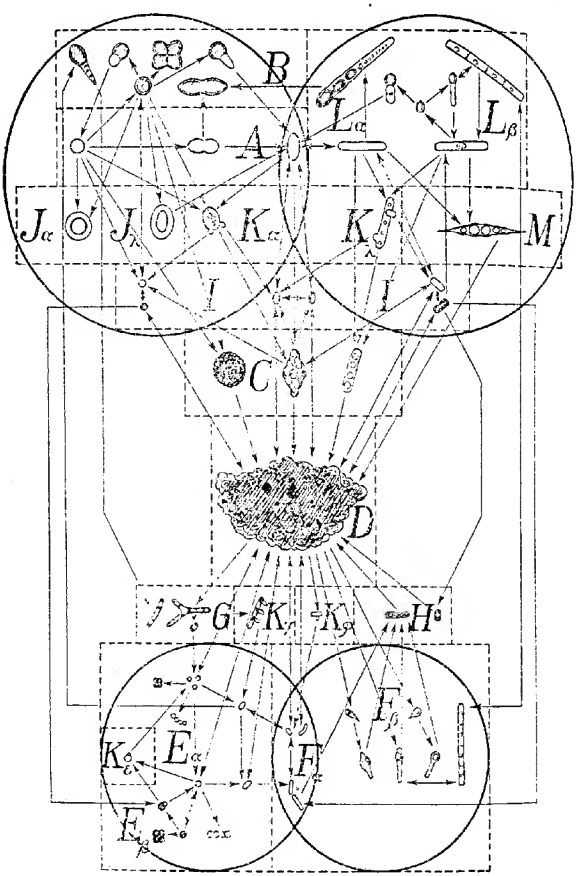


FIG. 1.—Life cycle of *Bacillus acetobutylicus*. The broken straight lines divide the different types of growth indicated by the letters A to M. The Greek letters α to λ refer to subdivisions. The single- and double-pointed arrows indicate the development of one form from another. The four circles confine, in every case, all those forms which represent together a rather constant mode of life and which have been usually considered as bases for establishing separate species.

fore so successfully dodged all bacteriologists, represents practically the key to a correct understanding of the whole problem. There are some

direct outside connections between the larger and the smaller forms, as indicated by the six arrows: I–E, E–B, B–G, and I–F, F–L, I–H; but they are rather rare exceptions. The symplastic stage D, on the other hand, can be observed in all cultures. Even if a strain shows little or no inclination to change from one subcycle into another, it regularly passes through type D. By frequent successive transfers (made each morning and evening), we tried to prevent this "breakdown." For some days we were successful, but then this disintegration again took place. After another couple of days the tendency to produce "normal" forms was once more very pronounced, which in its turn was again supplanted by the formation of type D. We have followed this rythmic alternation for some weeks with *B. azotobacter* as well as with *B. subtilis*.

It goes without saying that the arrangement and naming of the different types is merely a matter of convenience. Several of them perhaps could be split up into two or more. However, we found them so suitable in their present form for our work that we do not see any necessity for making an alteration.

Type A represents the normal, well-known, large Azotobacter cells of globular or oval form (usually 2 to 3μ broad and 3 to 5μ long). By further stretching they pass over into type I, (fig. 1 and 2 of Pl. A).

Type B indicates the thick-walled, rather resistant "arthrospores," regularly formed from type A.[1] When they germinate, either globular or oval forms are liberated. Some cells of type B, however, are produced occasionally by D, or still less frequently by a direct enlargement of small cells of type E or F, which will be discussed later. In the latter case the germ developing from B shows the character of type G. Probably no B formed by A germinates in this manner. This fact, then, would distinguish these morphologically identical forms.

Type C comprises all large forms in the stage of granular decomposition heretofore generally considered to be dying "involution" forms. If the granules are very small, they are nearly always easily stained. The larger ones, on the other hand, usually remain entirely unstained when treated with aqueous solutions of anilin dyes. Owing to the degenerated condition of the cell wall, the form of the cells frequently becomes quite irregular, and the granular content may become partially or entirely free (fig. 7 to 10 of Pl. B). Undoubtedly these granules are of different nature. Some may be fat, glycogen, or other metabolic products. Most of them however, are living entities, as is clearly shown by their further behavior, if not by their motility. Sometimes these granules develop to full-sized cells before being liberated. (See type J.) In this case they behave exactly like the "gonidia" in iron bacteria, as described by Cohn (4). However, in most cases they either leave the cell entirely before they

[1] We think it best to reserve the name "arthrospore" exclusively for those cases where the whole cell acquires the character of a spore. If only parts of the cell (either at its end or side) show such transformation, we call them "regenerative bodies" or "exospores," according to their special character.

start growing, or they grow out of it, piercing the cell wall. With all small bacteria we have observed only the two latter types of growth. Almquist (1), who made some similar observations, called these granules "conidia." In our opinion the term "gonidia"—that is, seed—is preferable, as these granules in every case act as organs of propagation and multiplication, whatever may be their special mode of growth.

Type D is in most cases the dissolution product either of the large forms (types C and M) or of the small cells (types E, F, and H), but it can also be formed by typical spores of type L, by regenerative bodies (I) and by gonidia. Its inclination to take the stain varies widely. If the cell walls participate considerably in its formation, it is readily and deeply stained. The same holds true when the gonidia are small and easily stainable. The large unstainable gonidia, on the other hand, which are frequently produced in type C, as well as in type H (see below), naturally give a rather pale or entirely unstained D. The structure, too, varies accordingly. Small cells, or small gonidia, cause a finely granulated, somewhat "hairy" structure; especially in the case of small slender rods like *B. fluorescens*, *B. radiobacter*, etc., the term "woolly" perhaps would be applicable. Large gonidia, on the other hand, as well as spores, clearly melt together when entering this stage of growth. Figures 7 to 12 (Pl. B), 18 (Pl. C), and 19 (Pl. D) illustrate the different possibilities. Like type C, type D has been considered by some investigators—for example, by K. B. Lehmann—as an occurrence indicating the death of the bacteria.[1] Usually, however, it has been passed as some uninteresting "slime" or "dirt." As it is made up by a thorough mixing or melting of a frequently large number of cells, spores, or gonidia, the term *symplasm* or *symplastic stage* seems to be a correct and convenient name for this stage.

Some time after the symplasm has been formed, very small granules, *regenerative units* (0.2 to 0.3μ), become visible. If the symplasm does not take the stain, the appearance of these organized well-stained forms inside the amorphous pale mass is very surprising (fig. 12 of Pl. B). Such a preparate indeed first turned our attention into this new direction. The regenerative units increase in size until they show the form of type E, F, I, or even A or B (fig. 13 and 14 of Pl. C). All these cells are easily stained, their cell walls being usually comparatively thick. At last, practically all the symplasm is reorganized, leaving sometimes only very few pale small "flakes."

Type E represents a miniature counterpart of types A and B. The size of the cells varies between 0.3 and 1μ. Only with the latter forms are the thin and the thick walled cells clearly discernible. In some cases at least, we were able to observe germinating arthrospores of this type. If necessary, both subtypes may be indicated conveniently by appending to the E a Greek letter α or β, respectively.

[1] The absurd name "zoogloea," which means "animal slime," has been repeatedly applied to this product of bacterial "autolysis," and the fact that the *walls* of the cells are dissolved has been considered as indicating the death of the *contents* of the cells.

Type F comprises all small rodlike cells of different form, straight or curved, about 0.3 to 0.5μ broad, 0.75 to 1.5μ, or more, long. When not forming spores, they may be labeled Fα; otherwise Fβ. Cells of the Fα type occasionally look very much like *B. radiobacter* and related species. In cases where great difficulties were encountered in getting a pure culture of *B. azotobacter*, this type of growth probably has repeatedly displayed an unwelcome activity. When developing from the symplastic stage, type Fβ shows different and somewhat unusual-looking intermediate forms (fig. 15 of Pl. C). For making a spore-bearing rod of the "Plectridium" type, a body splits off from the symplasm, showing a comparatively large "head" and a very small pointed "tail." When the tendency prevails, however, to form a "clostridium", the well-stained regenerative unit is located inside a pale sheath with pointed ends and in growing stretches until the albuminous substance is equally distributed inside the cell wall. Later, a part of the protoplasm once more concentrates, developing the spore. Spore-free, as well as spore-bearing, thin threads can directly, without passing through type D, change into the large type L. (See below.) On the other hand, they can also originate directly from this type (fig. 20 of Pl. D). In the latter case they sometimes resemble type G, from which they differ, however, by their spore formation and their genesis.

Type G shows, when treated with aqueous fuchsin, unevenly stained, frequently branched threads looking very much like Actinomyces. It is, however, as indicated in text figure 1 and shown in figure 16 (Pl. C), merely an intermediate step between types D and E which may be dispensed with. The small cells of type E are kept together by unstainable slime. Boiling water dissolves this slime within two minutes. A preparate treated accordingly with boiling aqueous fuchsin shows merely type E or some threads just dissolving (fig. 17 of Pl. C).

Type H acts as the counterpart of type C. There the larger, here the smaller cells are undergoing a granular decomposition leading to type D. However, rods, as well as spores, show a very unusual appearance in this case. They become entirely unstainable by aqueous dyes, but remain clearly visible even with a wide-open condenser, owing to the very bright luster of their granular content (fig. 18 of Pl. C).

Type I represents the globular, oval, or rodlike "regenerative" bodies which have been studied by Prazmowski (18). Here, again, an added α or β, respectively, may indicate their more or less resistant character (thin or thick cell wall). Iα usually originates from types A and B (fig. 5 of Pl. A) or from type C (fig. 7 of Pl. B). Iβ, on the other hand, in most cases is an offspring from the symplastic stage D (fig. 13 of Pl. C). Irregularly shaped type I, which is quite frequent with the other bacteria, has been observed only occasionally in cultures of Azotobacter (fig. 16 of Pl. C). The regenerative bodies either produce, by germinating or by stretching, cells of type A, B, or L or they convert themselves entirely into

regular spores. This possibility will be discussed at once. But, as mentioned before, regenerative bodies may also produce forms belonging to types E and F, making two of the "outside" connections between the upper and the lower circles as given in text figure 1.

Type J characterizes another rather rare occurrence also studied by Prazmowski. Forms belonging to type A, B, or occasionally L, increase in size and inside themselves develop one or more new full-sized cells of type A or B (fig. 6 of Pl. A and fig. 21 of Pl. D). These new cells are the result of the growth of the gonidia.

Type K comprises all those cells of type A, B, E, F, G, I, or L which produce one or more well-stained, round, oval, or rodlike buds, which, in the case of the large forms, occasionally cause a close resemblance to some budding yeast cell. These "buds" are gonidia developing into regenerative bodies, seldom directly into full-sized forms. An added α, β, ϵ, φ, γ, ι, or λ indicates the relation to type A, B, E, F, G, I, or L, respectively (fig. 3 to 6 of Pl. A; fig. 14, 16, 17 of Pl. C; fig. 20, 21, 24 of Pl. D).

Type L is made up of the large spore-free and spore-forming rods Lα and Lβ, as well as of free spores and long threads. Germinating spores of this type produce either long rods or rather short ovals resembling type A. The big spore-free rods and threads resulting from type A (fig. 1, 2 of Pl. A) seem to be unable to develop directly the faculty to form endospores. At least, we have never observed such a change, and this also would be in accordance with the fact that a direct transformation of a spore-free into a spore-forming bacterium has never been observed. As mentioned above, type Fβ, too, does not develop from type Fα, but directly from the symplasm. As is also noted above, these small spore-forming rods occasionally convert themselves into large spore-forming bacilli. Usually, however, the regenerative bodies formed by type D seem to be the normal predecessors. Under conditions, which will have to be studied more closely, these round cells acquire the tendency to produce endospores, which, in their turn, go back into the symplastic stage. This second symplasm then produces another set of regenerative bodies which stretch out to large granulated rods and threads. They later form the normal endospores.

Type M represents another rather unusual form. It originates from type L and passes over into type D (fig. 10 of Pl. B and fig. 19 of Pl. D).

It is hardly necessary to point out that sometimes our separation of the forms observed into different types becomes more or less arbitrary. For example, there are no absolutely sharp lines separating types A and B or the regenerative bodies I from the full-grown cells. Thick-walled cells of types E and F, when produced in the symplasm, might just as well be considered as "regenerative bodies." A germinating cell of type Eβ and a budding form of the type Kϵ resemble each other very

closely. However, similar difficulties occur in all such cases, where we want to reach some clear ideas concerning the multitude of the varying forms which we find in nature; and, as we now know that the life cycle of the same bacterium presents so many more different aspects than we ever expected before, we simply are compelled to take our refuge in some kind of classification, however crude it may be.

In Table I we give a summary of the different types of growth observed thus far in our preliminary studies with 24 representatives of the group of *B. azotobacter*. The laboratory numbers of the different cultures are to be connected in the following manner with the different types of Azotobacter, according to the denominations generally used:

No. 1, 2, 10, 11, 12, 14, 17 to 20, *Azotobacter chroococcum*, old stock cultures.

No. 21 to 25, *Azotobacter chroococcum*, new cultures isolated from different soils.

No. 3 to 6, 13 and 15, *Azotobacter Beijerinkii*.

No. 7 and 16, *Azotobacter vinelandii*.

No. 9, *Azotobacter vitreum*.[1]

TABLE I.—*Types of growth observed with 24 cultures of Azotobacter*

[The laboratory numbers of the cultures are given at the head of the columns]

Types of growth.	*A. chroococcum*, old stock cultures.		*A. Beijerinkii*.			*A. vinelandii*.	*A. vitreum*.	*A. chroococcum*, old stock cultures.		*A. Beijerinkii*.	*A. chroococcum*, old stock cultures.	*A. Beijerinkii*.	*A. vinelandii*.	*A. chroococcum* old stock cultures.				*A. chroococcum*, new cultures isolated from different soils.			
	1	2	3	4	5	6	7	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	+	+	+	+
B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–	–	+	+	+	+	+
C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
E	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
F	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
G	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
H	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
J											+													
K	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
M			+					+		+											+			

[1] Another strain of *A. vitreum* isolated in 1904 in Leipzig bore the No. 8 (now missing) in our collection. It died shortly before these investigations were started. One of the photographs (fig. 6 of Pl. A) was made by using an old preparate of this culture. We hope to be able to replace it later by a new subculture of the original strain left in the laboratory of the senior author in Leipzig.

More complete studies, of course, will fill all places now vacant in the table. The rather irregularly observed types G, H, J, and M are of no fundamental importance in the life cycle of *B. azotobacter*, and our interest has not been especially centered upon them. The large forms (types A, B, and L), as well as the small ones (types E and F), have been found in all cases. The same holds true concerning types D and I. This means that the full life cycle typical for *B. azotobacter* has been observed with every strain. That budding forms (type K) were also noticed in 23 out of 24 cases is of minor interest, because this is another type which represents no essential link in the cycle of bacterial life. Merely its unusual and heretofore nearly unknown appearance naturally attracted our attention.

The growth of the gonidia which causes this "budding" occasionally leads to three other kinds of development which deserve some short discussion. If the "buds" stretch out considerably, they cause the formation of branched bacteria, which can be found in the Azotobacter group, as well as in all other groups of bacteria. If, however, several gonidia, inclosed in the same cell or in its liberated granular content, start growing simultaneously in different directions, starlike forms result, which are frequently found in cultures of rodlike bacteria. *Bact. radiobacter* probably is the best known example of this special type of growth. But we have already reserved for a later publication good pictures of starlike outgrowths of slender rods from the typical large globular Azotobacter forms. An interesting crownlike form, representing the medium stage between simple budding and the formation of stars, is shown in figure 16 (Pl. C) directly above the upper symplasm. The third kind of gonidial development is another heretofore unknown type of growth. The budding gonidia sometimes develop into forms which clearly show the morphological and physiological character of typical endospores. Since they are produced, however, outside of the cell, they may be called *exospores*. In figure 20 (Pl. D) different stages of their development are reproduced. Their occurrence is not quite so surprising as it may seem to be at first, when considered in the light of the two following facts. Those "granules" which precede the formation of the normal endospores inside the bacterial cells are actually nothing else than small gonidia. When budding, the gonidia frequently develop into thick-walled regenerative bodies, which not only germinate in the same manner as endospores but may also acquire quite a considerable resistance against heat. As mentioned above, regenerative bodies growing out of the symplasm sometimes convert themselves practically entirely into spores. Budding exospores therefore are merely a special application of a general rule; they are regenerative bodies with the character of spores.

Normal heat-resistant endospores showing polar germination have been found in 13 of our Azotobacter cultures—that is, in more than 50

per cent of all cases. During our first investigations upon this subject (14) only 4 of 11 strains (No. 2, 4, 5, and 6) possessed this faculty, which they had acquired between 1908 and 1912. Culture 7 gave some bodies looking like endospores in 1914, but they were not resistant to heat. This faculty has now been fully developed. Cultures 15, 17, and 19, also typical spore-free cells at the time of their isolation some years ago, showed their inclination to form endospores when first tested in this direction in 1915. Cultures 23 to 25 developed this special character during the time we were experimenting with them. To fix exactly the conditions for transforming a spore-free into a spore-bearing strain will be one of the tasks in our later, detailed investigations. As already indicated, a close study of the symplasm and of the regenerative bodies derived from it will solve this problem as well as many others concerning the multitude of forms inclosed in the life cycles of the bacteria.

Unquestionably in all such experiments the inner condition of the cells is of no little importance. That at least in some directions this factor can eventually outmatch the influence of the outer conditions to a considerable extent is clearly shown by the interesting behavior of 22 of our Azotobacter strains when they were inoculated into soil extract containing 1 per cent of mannite and 0.05 per cent of monobasic potassium phosphate (KH_2PO_4), after having grown previously in moist sterilized soil and mannite. Fifteen of these cultures grew in the large types A and L and seven in the small types E and F when the experiment started. Two weeks later, without a single exception, all the fifteen strains changed from types A, B, and L to D, E, and F. Vice versa, the seven others produced types D and I, these developing into types A, B, and L. This result corroborates once more our statement that every strain of Azotobacter or of any other bacillus will pass through all phases of its cycle of life persistently if the conditions are suitable. Undoubtedly and in full accordance with the behavior of higher organisms, some strains are especially inclined to grow mostly in one or the other subcycle. However, the formation of the symplasm and its "plasticity" enables us, if we make use of these interesting possibilities, to induce and accelerate changes in the general development of a bacillus to which the special strain perhaps may be only very little inclined at that time. For example, our five newly isolated Azotobacter cultures 21 to 25 had, of course, the pronounced tendency to grow in their typical large globular or oval form. Only in about 1-month-old mannite solutions were the long rodlike forms more numerous, mixed with forms belonging to types A, B, D, E, F, and I. Transfers on mannite agar, after one day, gave in four cases an abundant and practically pure growth of the large rods, showing a tendency to form endospores. Only one culture (No. 24) failed, because in this case we had no such old solution at hand and had started, therefore, from a 3-day-old culture, which exclusively produced round and oval regenerative bodies on mannite agar.

Mannite soil extract, which has been used with very satisfactory results by the senior author (13) for more than 10 years for the study of nitrogen-fixing and other soil organisms, unquestionably has the disadvantage of a varying and partially unknown chemical composition. However, beef broth and similar substrates are liable to the same objection and yet are generally used in bacteriological laboratories. Nevertheless it would be preferable to have media at our disposition of the same favorable qualities but of well-defined chemical composition. Practically all of the many artificial substrates recommended in the bacteriological literature are much too concentrated, especially for soil organisms. For many of our experiments we used the following mineral solution to good advantage:

Monopotassium phosphate neutralized to phenolphthalein by sodium hydroxid	0.02 per cent
Magnesium sulphate	.02 per cent
Sodium chlorid	.02 per cent
Calcium sulphate	.01 per cent
Ferric chlorid, 1 per cent solution	2 drops per 100 c. c.

As carbonaceous material for *B. azotobacter*, 1 per cent mannite was used. The further addition of 0.02 per cent of potassium nitrate or peptone proved to be beneficial though not necessary. All these solutions are entirely clear and therefore especially suitable for microscopical studies. If a strip of filter paper sufficiently long to reach about 1 inch out of the solution is placed into the test tube before sterilizing, a luxuriant growth of the large forms belonging to types A and B quickly spreads on the part above the liquid.[1] In old solutions the symplasm frequently develops to such a degree that it becomes clearly visible to the naked eye as white flakes or slimy threads. Figure 14 (Pl. C) is a reproduction of the end of such an enormous accumulation of living material.

The last three pictures of our Azotobacter series (fig. 22 to 24 of Pl. D) illustrate one of the comparatively rare direct connections between the small and the large forms. We have here before us the exact counterpart of the alteration shown in figure 20 (Pl. D). Certainly this direct growing up of the small organisms to forms belonging to type B, the forthcoming of threads of type G in the germinating process, and the unusual appearance of their budding, by which the small forms are regenerated, deserve our full attention. However, this kind of development seems to be a rather rare exception to the rule. These forms also are much inclined to turn into the symplastic stage. A photographic picture of this occurrence will be published later.

The close study of this side connection, however, led us to another discovery which we had failed to make before, although our other preparates,

[1] The arrangement mentioned above is very helpful for obtaining pure cultures of Azotobacter from the soil. At the same time it allows the motility of an organism to be determined macroscopically. One of our strains crept up 10 cm. in 10 days on long paper strips in large test tubes.

as reproduced in figures 1 to 3 (Pl. A) and 8 (Pl. B), show the same fact much more clearly. In all these preparates many cells are in a conjunct stage, which can not be explained by the assumption that this *conjunction*[1] is only accidental. Most of these illustrations have been made from contact preparates taken directly from 4-day-old colonies. Smears made in accordance with the "standard methods" probably would have destroyed most of these connections. But also in this case it was still more the effect of our theoretical blinders which prevented an earlier seeing and understanding of this fact, which, like the budding of the bacteria and the formation of the symplasm, has not only actually been seen by many bacteriologists but also has been unknowingly reproduced in several illustrations in our daily used textbooks.

So far as we are aware, only one author has spoken of a similar observation. In 1892 Förster (6) found occasionally that *Chromatium Okenii* sometimes entered into some "primitive copulation." Among the drawings accompanying his paper, a sketch made from a photomicrograph seems to us most trustworthy. Its conformity with our Azotobacter illustrations is practically complete. Observations in the hanging-drop clearly showed that there is some interference between the plasmatic substances in the conjunct cells or even some direct mixing of them.

The determination of the actual physiological significance of this conjunction must be left, of course, to a more thorough investigation. At present we merely wish to add and to emphasize that this process is by no means such an exception as might be deduced from Förster's statements and from the silence observed in this direction in our textbooks. The conjunct stage seems to be of no less general importance and occurrence in bacterial life than the formation of the symplasm. Not only normal cells and regenerative bodies but also exospores have been frequently found in conjunction. And if we only succeed in forgetting for a moment our most cherished theories and simply try to look at the facts as they are, we find at once that the formation of the symplasm and the conjunction of the cells are nothing else than two modes of mixing plasmatic substances temporarily inclosed in separate cells and that evidently the continuity and rejuvenescence of the living matter in the bacteria is just as much dependent on this process as in the case of all other organisms.

A thorough study of the relations existing between the conjunct and symplastic stage will be the first object of our further investigations in this line. We hope that experiments with well-defined varieties and species will soon furnish a correct insight. The ease with which the "flakes" of the symplasm can be isolated is, of course, very advantageous for these, as well as for systematic, studies.

[1] We prefer the new term *conjunction* instead of "copulation" or "conjugation," because frequently more than two cells unite and no sexual differentiation so far has been observed.

Before entering a discussion of the life cycles of other bacteria, the serial numbers for the four subcycles of *B. azotobacter* may be given, determined, so far as possible, in accordance with the methods recommended by the Society of American Bacteriologists. The behavior in the presence of the different carbonaceous substances, however, had to be tested in our mineral solution with nitrate to which 0.5 per cent of the different sugars, etc., was added, the highly concentrated peptone solution not being suitable for this organism. That the appearance of the colonies, as well as the other cultural characteristics, differs accordingly, goes without saying; these details will also be given later. The serial numbers resulting from our tests are as follows:

Type A.—221.2322813. Type E.—222.2222524.
Type L.—121.3332033. Type F.—122.4442034.

THE LIFE CYCLES OF OTHER BACTERIA

The following 18 cultures were selected as representatives of practically all groups of bacteria.

No. 31. *B. subtilis*, isolated from evaporated milk.
No. 32. *B. lactis niger*, Gorini's original culture from Kral's Museum.
No. 33. *Tyrothrix tenuis*, Duclaux' original culture from Kral's Museum.
No. 34. *B. danicus*, isolated from soil.
No. 35. *Bact. pneumoniae*, isolated from soil.
No. 36. *Bact. radiobacter*, isolated from soil.
No. 38. *Bact. denitrificans agile*, Ampola's original culture from Kral's Museum.
No. 39. *Bact. radicicola*, isolated from vetch.
No. 40. *Bact. fluorescens*, isolated from milk.
No. 41. A yellow bacillus (not determined) isolated from soil.
No. 42. *Planosarcina ureae*, Beijerinck's original culture from Kral's Museum.
No. 43. *Sarcina flava*, isolated from milk.
No. 44. *Micrococcus candicans*, isolated from chernozem.
No. 45. *Micrococcus candicans*, isolated from evaporated milk.
No. 46. Salt-water spirillum isolated from Great Salt Lake, Utah.
No. 47. Salt-water spirillum isolated from sea water.
No. 48. *Streptococcus lactis*, kindly furnished by Dr. L. A. Rogers, Bureau of Animal Industry.
No. 49. *Bact. bulgaricum*, kindly furnished by Dr. L. A. Rogers.

Before being tested, these cultures had been grown on the following substrates:

Beef agar: No. 31–36, 38, 40, 41, 43–45.
Beef agar plus 3 per cent of sodium chlorid: No. 46, 47.
Beef agar plus 0.5 per cent of urea: No. 42.
Saccharose agar: No. 39.
Milk: No. 48, 49.

After having been examined as to their purity on agar plates, they were cultivated on the different agars and in suitable solutions. Beef, salt, and urea agars were used as before. In the case of *B. radicicola* (No. 39),

however, the saccharose agar was substituted by mannite agar as prepared for *B. azotobacter*. The milk organisms (No. 48 and 49) were transplanted on yeast-whey agar and into yeast-whey solution, these media being the most suitable for these organisms, according to the earlier observations of the senior author (13). The salt-water spirilla were grown in beef broth with 3 per cent of sodium chlorid. For the other organisms various solutions were prepared by adding to the mineral solution as used for *B. azotobacter* the following ingredients:

0.1 per cent of ammonium citrate plus 0.3 per cent of glycerin for cultures 31, 33 to 36, 38, 40, 41, 44, and 45.
0.04 per cent of peptone plus 0.3 per cent of glycerin for cultures 32, 42, and 43.
0.02 per cent of potassium nitrate plus 1 per cent of mannite for culture 39.
0.04 per cent of peptone plus 0.5 per cent of lactose for cultures 48 and 49.

All cultures were kept at 28° C. with the only exception of those of *Bact. bulgaricum* (No. 49), for which a temperature of 40° to 45° C. is more suitable.

In the light of the results obtained by us with *B. azotobacter* it was not difficult to find out that the life cycles of all these organisms are essentially the same. On all good substrates they all pass quite regularly through most, if not all, of the types of growth first observed with *B. azotobacter*. In Table II the results are summarized. Type N, which we have added here, represents the starlike growth previously mentioned.

TABLE II.—*Types of growth observed with 18 representative bacteria*

[The laboratory numbers of the cultures are given at the head of the columns]

Types of growth.	*B. subtilis*	*B. lactis niger*	*Tyrothrix tenuis*	*B. danicus*	*Bact. pneumoniæ*	*Bact. radiobacter*	*Bact. denitrificans agile*	*Bact. radicicola*	*Bact. fluorescens*	Yellow bacillus	*Planosarcina ureæ*	*Sarcina flava*	*Micrococcus candicans* (soil)	*Micrococcus candicans* (milk)	Salt Lake spirillum	Ocean spirillum	*Streptococcus lactis*	*Bact. bulgaricum*
	31	32	33	34	35	36	38	39	40	41	42	43	44	45	46	47	48	49
A (large globules and ovals)	+			+						+	+							
B (thick walled forms of type A)	+	+	+	+						+	+							+
C (granular decomposition of A, B, L, M)	+	+	+	+						+	+							+
D (symplasm)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
E (small globules and ovals)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
F (small rods and threads)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
G (slime threads with cocci)		+	+	+	+	+	+	+	+	+	+	+					+	+
H (granular decomposition of F and spores)	+	+	+	+	+	+	+	+	+	+	+		+					+
I (regenerative bodies)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
J (normal cells developing inside)	+		+	+						+								
K (budding gonidia)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L (large rods and threads)	+	+	+	+						+	+							+
M (cells with pointed ends)						+	+	+	+				+	+			+	+
N (starlike growths)	+	+			+	+	+	+	+	+			+	+	+			+

The disintegration of the normal cells into the symplastic stage (D), the formation as well as the further development of regenerative bodies

(I), and the occurrence of gonidia budding out of the normal cells (K) have been observed in every case. With all those cultures which regularly produce endospores (No. 31–34, 41, 42), large cells belonging to types A, B, C, and L were observed; and they were not observed with the same constancy in cultures 35–40, 43–48. Of all these organisms none are known to produce endospores. *Bact. bulgaricum* (No. 49), too, is spore-free; but closely related forms isolated from the stomach have been reported as producing endospores. The formation of cells of type B, C, and L makes it highly probable that an experimental trial to induce spore formation may soon be successful. However, the same possibility is by no means excluded in the other cases. It may be that small spore-forming forms can be branched off from the other cultures. Indeed, we have already obtained some quite encouraging results in this line. Whether, then, another progression to the large cell types will be possible is entirely an open question.

Referring again to our introductory remarks, we take this opportunity to point out specifically that these perhaps somewhat surprising statements should by no means be considered merely as some absurd polymorphistic hypothesis. The well-known character of *Bact. pneumoniæ*, for example, will by itself remain completely unchanged, whatever may be the result of further investigations upon the full life cycle of this organism. If there is a spore-forming type, and perhaps even genetic relations with some large-sized cells, this would in no way interfere with nor impair the well-established facts already collected. Such wide morphological differences must always be connected with no less considerable alterations of the whole physiological character, so that these other types, if they are known, of course, are stored away as entirely different "species" in various remote places in the so-called "system" of bacteria. This conclusion can be drawn with absolute certainty from our observations on *B. azotobacter* as well as from Henri's experiments (10) with *B. anthracis*. If only those changed forms, frequently seen in all bacteriological laboratories, had not been persistently discarded as uninteresting "involution forms" or as "contaminations," the whole situation would undoubtedly be much clearer and much more satisfactory. At present it is not our intention to dwell upon the numerous details collected in our studies of the life cycles of the different organisms. Though the broad types of growth are the same with all, the morphological details, of course, differ considerably. Figures 25 to 30 (Pl. E), 31 to 36 (Pl. F), and 37 to 40 (Pl. G) will furnish sufficient proof in this direction, especially when compared with our illustrations of *B. azotobacter*. It may suffice to add the following remarks:

Figures 25 to 27 (Pl. E) illustrate the appearance of the same cultures of *B. subtilis* on a beef-agar slant, made from a 1-day-old colony on a beef-agar plate. The smear made directly from the colony showed

the typical spore-forming rods as reproduced in the atlas of Lehmann and Neumann (11, pl. 47, fig. V). Figure 25 (Pl. E) was made from a preparate from a 2-day-old agar slant. Spores dissolve into stage D; and thick-walled, more or less irregular regenerative bodies are being formed. This process is going on in the 6-day-old culture (fig. 26 of Pl. E). The "melting" of the spores is clearly visible. The regenerative bodies have increased in number as well as in size. Some forms resemble very much those of *B. radicicola*. After eight days (fig. 27 of Pl. E) these regenerative bodies are either dissolved entirely into a readily stainable symplasm or they produce bright granulated spores (type H), which later also pass over into the symplastic stage. Sometimes the unstainable content of the regenerative bodies slips out of the dark-stained cell wall, forming an unstainable symplasm like that frequently produced by cells of the C type of *B. azotobacter* (fig. 6 of Pl. A; fig. 7 of Pl. B. See also the mixture of stained and unstained symplasm in fig. 19, Pl. D). The new set of regenerative bodies developing from the symplasm, especially from the dark-stained material derived from those irregular forms, usually showed rodlike forms stretching out into long granulated threads, which, in their turn, divided themselves into the normal spore-forming rods typical of *B. subtilis*.

This behavior was observed, only slightly modified, with all cultures of spore-forming rods. Figure 28 (Pl. E) shows this regerneration of the new threads from the symplastic stage as it was found in a 2-day-old transfer of the "yellow bacillus" (No. 41), made from a 12-day-old peptone-glycerin solution into the same liquid substrate. The thread on the right side of the field illustrates the situation especially well. As the upper part is broken off, the gonidia inside the cell, which caused the formation of the short branch on the lower part, become visible. The symplasm still contains several regenerative units which apparently are checked by the vigorous absorptive action of the thread.

The special appearance of many types of growth of *Bact. bulgaricum* is plainly discernible in figure 29 (Pl. E), made from a 6-day-old stab culture in yeast-whey agar. Large and small rods (types L and F), pointed forms (type M), the formation of regenerative bodies (type I) budding (type K) are clearly visible. On the left side of the figure two long, thin threads grow ("branch" or "bud") out of the same point in a larger rod. Below this another thick rod, showing granular decomposition (type C), is reproduced. In the middle of the field some thin, pale symplasm (D) is spread out. Above, a thin pale thread containing darker stained bodies (type G) crosses the field. Some small cells of type E are lying close to it. That the round cells budding out of the rods are indeed regenerative bodies is proved by their germination, the new rods growing out in one or in two directions. This frequently happens when the regenerative bodies are still connected with their mother cell.

The formation of the symplasm in an 11-day-old ammonium-citrate-glycerin solution of *B. fluorescens* is shown in figure 30 (Pl. E). This figure should be compared with figure 11 (Pl. B), showing the formation of stained symplasm of *B. azotobacter*. The dark rods also visible in the former figure are of the H type.

In figure 31 (Pl. F) cells of *Sarcina flava* from a 1-day-old beef-agar slant are reproduced partially disintegrating into the symplastic stage. The small symplasm in the center has already entered the formation of regenerative units. Many of the cells are in the conjunct stage.

Figure 32 (Pl. F) illustrates the transformation of the symplasm of *Streptococcus lactis* into many normal forms and some round regenerative bodies, as observed in a 5-day-old peptone-lactose solution. As far as this transformation has already taken place, it is clearly discernible that indeed, as mentioned before, the whole material is used again for the reproduction of new cells practically without leaving any remnants. Figure 33 (Pl. F) shows another "flake" of symplasm of *Streptococcus lactis* from a 3-day-old milk culture containing many regenerative units and some globular regenerative bodies. This illustration is of special interest for the following reasons: Such globular bodies of different diameters are produced by all kinds of bacteria (cf. fig. 13 of Pl. C; fig. 25, 26, 27 of Pl. E). If they are dispersed in their symplasm and this is embedded in the equally deeply stained casein of the milk, it looks nearly as if the albuminous substances of the milk were forming granules which later produce normal bacteria by germinating or stretching. The center of figure 33 (Pl. F), where a rather compact symplasm is lying above a very thin film of casein, shows that these things are entirely separate and different. However, in the lower left part of the field some symplasm is embedded in a thicker layer of casein, and here the situation is much less clear. Now, Fokker (5), one of the few authors who are still fighting in favor of spontaneous generation, has repeatedly pointed out that his standpoint is strongly supported by the fact that the albuminous substances in animal tissues, as well as in milk and in blood, produce small granules which later develop into normal bacteria. The assumption that his subtrates were not sterile, of course, does not furnish a complete explanation of these peculiar observations. We believe, however, that our discovery of the symplasm and of its regenerative units settles this question.

That the formation of the symplasm and the regeneration of new cells are by no means an abnormal occurrence merely caused by the unnatural conditions under which our cultures are compelled to live in the laboratories can be deduced without great difficulty from different facts already mentioned. However, we thought it useful to add to the illustration of the milk culture another one reproducing an entirely "natural" occurrence. Figure 34 (Pl. F) was taken from a smear made directly from the content of a root nodule of red clover. The irregular, frequently branched

large forms[1] are passing over into the symplastic stage. Many bright gonidia, some deeply stained regenerative bodies, and a few normal slender rods are seen. This illustration should be compared with figures 26 and 27 of Plate E.

The two salt-water spirilla included in our experiments were also inclined to produce symplasm, globular and irregular regenerative bodies like the representatives of all other groups of bacteria. We have preferred, however, to show in figures 35 and 36 (Pl. F), which were made from a salt-beef-agar slant only 4 hours old, some facts which confirm and explain two observations made several decades ago. In 1887 Sorokin (19) published a preliminary communication upon his "new species" *Spirillum endoparagogicum*, of which, so far as we know, a full description has never been given. His illustrations, reproduced in several textbooks, show clearly that he also found a budding bacterium without becoming aware of this fact. That the bright granules contained in the large spirilla and budding out of it, forming new small rods and spirilla, were not endospores, as the author asserts, seems to be beyond question. No test was made of their heat resistance, and in our opinion the fact that many of them were produced in the same cell proves sufficiently that they were gonidia. Their globular form is also much more in agreement with this opinion than with the assumption that they were endospores. Figure 35 (Pl. F) shows the same budding of our salt-water spirillum. Many of the irregular "involution" forms, so frequently observed with other spirilla, belong also to this type of growth. Furthermore, in figure 35 (Pl. F), as well as in figure 36 (Pl. F), several round regenerative bodies are reproduced, some of them being in the germinating stage. They are either dark-stained like those of other bacteria or they remain unstained when treated with aqueous anilin dyes. If such unstained forms are budding out of the end of a spirillum, as can be seen in the center and at the right side of figure 35 (Pl. F), we have apparently before us the same occurrence which was described by Prazmowski in 1880 (17, p. 43). We have not yet tested the heat resistance of these bodies. It is possible that they are parallel forms of the exospores found in the spore-forming L type of *B. azotobacter*. In the meantime they may be registered as unstained regenerative bodies. Some different types of germination are exhibited by the three regenerative bodies in figure 36 (Pl. F). The lower right part of figure 35 (Pl. F) contains several spirilla which may be in the conjunct stage. They are wound closely around each other, forming apparently one thick cell, only the end parts being separated. An analogous occurrence with *Spirochaeta obermeieri* has been recently observed by Levy

[1] They have been called "bacteroids" by Brunchorst because this author conceived the wrong idea that they were not bacteria, but cell products looking somewhat like bacteria. We are unable to understand how such an entirely incorrect term can still be used in modern scientific publications.

(12), who is also of the opinion that some "copulative" process takes place.

In future publications we will have to give more illustrations showing the different forms of the various kinds of regenerative bodies produced either directly by the different bacteria or by their symplasms. It seems as if such irregular, sometimes monstrous-looking formations as reproduced, for example, in figure 26 (Pl. E), are very constant and very characteristic for the species to which they belong. This is already well known in the case of *B. radicicola*, and our figures 37 and 38 of Plate G may demonstrate the same fact in relation to *Micrococcus candicans*. The preparates were made from 6-day-old cultures in an ammonium-citrate solution. One strain (No. 44) had been isolated about six years ago from Russian black soil; the other (No. 45) nine years ago from evaporated milk. The characteristic appearance, as well as the uniformity of both illustrations, deserves our full attention.

Figures 39 and 40 (Pl. G) show the formation of well-stained gonidia by the yellow bacillus (No. 41) and by *B. fluorescens* (No. 40). In both cases the transformation of gonidia into regenerative bodies is clearly visible (cf. fig. 29 of Pl. E). Figure 39 (Pl. G) contains also in its lower left quarter some germinating regenerative bodies. The "budding" process is very conspicuous in this case, but the second picture makes it clear that the size of the gonidia frequently becomes so small that even a very careful observation of the stained preparate is hardly of any use. Besides, as many of these gonidia do not take the stain at all, some filtering experiments and the observation in the dark field seemed to be preferable.

FORMATION OF FILTERABLE GONIDIA

The formation of gonidia has been observed with all our different cultures, but whenever we saw a large number of gonidia in our preparates, there were always some, frequently many, just at the limit of visibility. It was to be expected that these would pass through Chamberland bougies. As our other experiments had shown that these gonidia are indeed living entities, some tests seemed to be of interest, especially in view of the many open questions concerning the occurrence and character of filterable vira.

The following cultures were used for making filtering tests:

No. 1 (*B. azotobacter*), 24 days old in a mannite-nitrate solution; No. 31 (*B. subtilis*), 11 days old, in ammonium-citrate-glycerin solution; No. 33 (*Tyrothrix tenuis*), No. 35 (*Bact. pneumoniae*), and No. 40 (*Bact. fluorescens*), each from a 2-day-old culture in ammonium-citrate-glycerin solution.

The filtrates were first tested under the microscope. Stained preparates had to show the absence of large forms. By the use of the dark field the small gonidia could easily be seen, some of them being actively

motile. These filtrates were then transferred to beef agar, beef broth, milk and blood serum. After incubating, the macroscopical appearance was the same in all cases. On the agar slope, especially on its lower moist part, a very scant, thin, slimy growth, somewhat resembling a very thin layer of small droplets of dew, became visible. A stained preparate from a 4-day-old slant clearly showed the gonidia germinating to minute rods (fig. 41 of Pl. G). The other substrates also gave no conspicuous growth.

The dark field proved more efficient in observing these almost invisible forms. Figure 42 (Pl. G), made from the same 4-day-old slant as figure 41, shows clearly that the filterable gonidia also form a symplasm in the same manner as the larger ones, which, in its turn, produces new small cells. In order to bring out more definitely the structure of this symplasm, it was necessary to make a very dark print thereby obliterating several free granules which were also visible in the field.

These facts are in good agreement with the observations of various authors concerning filterable vira. In one of the latest publications along these lines Healy and Gott (9) described the filterable organism causing hog cholera as small globules or rods (0.2 to 0.3μ) when growing on ordinary media appearing in slimy clumps which are either well stained with aqueous dyes or are not stained at all.

As a regeneration of larger forms could in no case be observed on the subtrates mentioned, we also transferred small quantities of filtrates of cultures 31, 35, and 40 into ammonium-citrate solution. Here a quick regeneration took place. After two days some sediment was already formed, which after shaking caused a distinct turbidity of the solution. Under the microscope in the stained preparate many pale, stained, small granules and minute rods were visible, as before, and also larger dark stained oval forms 0.5 to 1μ broad, 0.75 to 1.5μ long. These forms still differ considerably in their appearance from the normal rods of *B. subtilis* or *Bact. fluorescens*. They may be classified as regenerative bodies. That they will turn back entirely to the normal large vegetative cells is not doubtful, but this still remains to be tested experimentally. So far our tests have been repeated three times with *Bact. fluorescens* and twice with *B. subtilis*. The results were identical. For the filtration we used three different filters, which were controlled in each case by obtaining sterile filtrates from 1-day-old cultures of *B. subtilis* or *B. azotobacter* 1.

In carrying out experiments like these, however, another possibility of obtaining erroneous results must be kept in mind. Not only must the media be carefully prepared and sterilized but all glassware must be thoroughly treated with some cleaning fluid such as chromic acid, which destroys entirely all bacterial forms. The fact that mere sterilization is not sufficient is shown by the following test:

Particles of symplasm containing many regenerative bodies were carried from a mannite-nitrate solution to a similar medium and heated

in the autoclave for half an hour at 20 pounds' pressure. Even after this harsh treatment the microscopical picture was practically unchanged. As substrates rich in organic matter, such as beef agar, frequently contain symplasm and regenerative bodies resulting from former bacterial growth, they are especially liable to give misleading results.

DISCUSSION OF RESULTS

We hope that the facts mentioned in this preliminary communication will suffice to awake an adequate interest among our fellow bacteriologists, as there are numerous problems which now can be attacked successfully from this new standpoint. It is true that several authors before us have already spoken of the "life cycles" of bacteria. In most cases, however, they meant only the straightforward (not "cyclic") development, consisting in stretching and dividing of the cells, sometimes combined with the formation and germination of endospores. Fuhrmann (7, 8), who also wrote upon the "*Entwicklungskreise*" of bacteria, made some correct observations concerning the formation and further development of the gonidia. He was wrong, however, in concluding that these "granula" which he found in some spore-free bacteria were practically counterparts to the endospores in the "life cycle" of the spore-forming bacilli, and his opinion upon the "detritus" resulting from the disintegrating cells—namely, the symplasm—was far from being correct. In this direction Fokker (5) came much closer to the truth. It is not impossible, of course, that by a thorough sifting of the literature we shall discover some entirely forgotten author who was already on the right track. So far as we know now, only one bacteriologist has previously seen all the different stages of growth typical of the full life cycle of the bacteria. We refer to De Negri's important "*Untersuchungen zur Kenntnis der Corynebacterien*" (16), which appeared this spring, when we had just begun to prepare this paper for publication. A comparative study of the illustrations of his article and those of the present paper will be very instructive. He registered the following forms produced by the organism which causes the "malignous granulom:"

Large globules (2.5 to 5.5μ) sometimes in sarcina form, eventually developing round or rodlike germs or buds ..... Our types A and B
Large forms containing granules occasionally unstainable ..... Our type C
Crumbly agglomerations formed by large forms "melting" together, which later give birth to new small forms ..... Our type D
Small globules frequently in chains ..... Our type E
Small short rods (¾ by 1μ), small slender rods (¾ by 1½ to 2μ), rods containing granules, curved rods, and rods showing racket form ..... Our type F
Granulated threads dissolving into small globules ..... Our type G
Entirely unstained bright rods ..... Our type H

Globular forms of different size sometimes showing a thin protruding rodlike form, irregular curved or clublike forms which later produce normal rods.....Our type I
Budding large globules, budding and branching rods and threads.....Our type K
Large rods and threads.....Our type L
Pointed rods containing large granules.....Our type M

This complete agreement is indeed very interesting, and as we ourselves have not worked with any representative of this group of organisms, De Negri's observations furnish a very welcome extension and confirmation of our statements concerning the life cycles of all bacteria. De Negri himself unfortunately failed to see that he was touching this general problem. He confined his studies almost exclusively to those corynebacteria causing "malignous granulom" and to some closely related forms. Therefore he was carried away to the entirely incorrect conclusion that those large budding forms were some kind of "blastomycetes," and the organism studied by him should be separated from the bacteria and placed among the Fungi Imperfecti. A comparative study of any of the common bacteria—for example, *B. subtilis*—would easily have prevented this serious error.

For *diagnostic* and *systematic* purposes a full knowledge of the life cycles of the bacteria will naturally be of the greatest importance. In our opinion the following morphological details should be studied in every case.

1. VEGETATIVE CELLS; FORMATION AND GERMINATION OF SPORES
   - Spore-free and spore-bearing cells
   - Arthrospores, formation and germination
   - Endospores, formation and germination
   - Exospores, formation and germination
2. CONJUNCTION OF DIFFERENT CELL TYPES
3. GONIDIA, formation and development
   - Budding, liberating, germination, development in toto to regenerative bodies, to exospores, or to full-sized cells
4. SYMPLASM, formation by
   - Spore-free cells
   - Spore-bearing cells
   - Arthrospores
   - Endospores and exospores
   - Regenerative bodies
   - Gonidia
5. REGENERATIVE BODIES
   - Formation by
     - Spore-free and by spore-bearing cells
     - Arthrospores, endospores, and exospores
     - Gonidia of different types
     - Symplasm of different origin
   - Germination of the different types
   - Development in toto to vegetative cells or to spores

The improvement of the present situation is obvious. As the full life cycle of probably every species of bacteria can be studied without difficulty within a few weeks, provided suitable media are known and used for the experiment, we may hope that the time of reckless species-making will soon be ended. As said before, "good" species will win very much by such renewed and thorough study. The innumerable others, however, will have to take their modest place as links in those life cycles to which they really belong, or they will have to be canceled entirely. That the discovery of the conjunct and symplastic stage and further experimental studies upon it are of fundamental importance for reaching correct conclusions concerning species or varieties is beyond question.

Undoubtedly all our *physiological* studies will gain in much needed conformity and accuracy when established on the new broad morphological basis. It is to be hoped that such investigations now will also meet with more interest in botanical laboratories, where many of the general problems in bacteriology should be studied, as usually the time of agricultural and medical bacteriologists is completely taken up by their more specialized work. For instance, those curious but heretofore entirely unexplainable regular seasonal variations in the activity of bacteria in soils, quite frequently observed in Europe as well as in America during the last years, now seem to become explainable as a result of the seasonal effect upon the different modes of multiplication and propagation of the bacteria. A similar dependency on this factor then would exist as with other organisms. At least we can hardly consider it being merely an accidental coincidence that essentially the same annual curve, showing a maximum in spring and another one in autumn, is also followed by lower fresh-water algæ, where, as Transeau's careful investigations (20) have shown, the temporary prevalence of spore formation and of vegetative processes apparently represents the principal cause of these variations.

Concerning *pathological* problems, we readily admit that we are entirely laymen. However, we feel sure that this branch of bacteriology also would win considerably by making use of our observations. They show that Henri's (10) very interesting results obtained with *B. anthracis* could easily be duplicated with this or other pathogenic species simply by studying the relation of virulence and type of growth. That those abnormal looking and abnormally reacting forms obtained by the French author by the application of ultra-violet rays are nothing else than some of the regular though heretofore unknown types of growth of *B. anthracis* needs hardly be emphasized. Investigations upon the relations existing between pathogenic and nonpathogenic bacteria, as well as the experimental transformation of one type into the other, now undoubtedly become much more accessible and promising. The same holds true concerning the filterable vira. At least some of them are surely to be explained as nothing else than filterable gonidia of well-known bacteria.

That the discovery of the complete life cycles of the bacteria solves also some problems in *general biology* has been indicated earlier in this paper, when Fokker's theory (5) concerning the development of bacteria from granules in milk or blood was discussed. It may be added that also those much doubted and disputed strange observations of Bastian (2, 3), so persistently and extensively defended by their discoverer, now are coming under entirely new aspects. Readers interested in this question should compare especially Plates IV and V of Bastian's "Nature and Origin of Living Matter" (2) and those on Plates X and XI of his "Evolution of Life" (3) with our illustrations of the different kinds of symplasm and regenerative bodies. Figure 33 of the last-named plate (XI) looks practically like a reproduction of our preparate shown in figure 14 (Pl. C). Bastian was wrong, of course, when he considered those large cells as being some torula form; but we know that De Negri (16) made the same mistake recently, which indeed is quite excusable. That the budding large cell in our figure 14 is really nothing else than a type of growth of a spore-forming bacillus will probably even now be doubted by one or the other bacteriologist. It is superfluous to point out that we do not share Bastian's ideas concerning abiogenesis. Our standpoint in this case is the same as in regard to Fokker's hypotheses. The weak points in Bastian's experiments are sufficiently clear to every expert reader of his books. This, however, should not lead to discarding indiscriminately all his undoubtedly carefully made microscopical observations.

It goes without saying that we will readily furnish subcultures of the strains used in our studies to everyone who asks for them. But it probably would be still more interesting and surprising to our fellow bacteriologists if they would make some investigations with their own well-known stock cultures along the lines discussed in the foregoing pages. Even a renewed microscopical study of old stained preparates may become very instructive. For example, the senior author also did not know that for more than 11 years he had in his collection, patiently waiting to be photographed, that fine preparate now shown in figure 6 (Pl. A) until, as stated before, he decided to take down his "theoretical blinders." We have already mentioned that a careful study of the illustrations contained in our daily used textbooks will now reveal several things which we were so very well trained not to see. Certainly the German philosopher Lichtenberg made a very wise remark when he said:

Was jederman für ausgemacht hält, verdient am meisten untersucht zu werden.

SUMMARY

A comparative study of 42 strains of bacteria has shown that the life cycles of these organisms are not less complicated that those of other micro-organisms. As representatives of practically all groups of bacteria have been tested and all, without exception, behaved essentially in the

same manner, in all probability analogous results may be expected with all species of bacteria.

All bacteria studied live alternately in an organized and in an amorphous stage. The latter has been called the "symplastic" stage, because at this time the living matter previously inclosed in the separate cells undergoes a thorough mixing either by a complete disintegration of cell wall, as well as cell content, or by a "melting together" of the content of many cells which leave their empty cell walls behind them. In the first case a readily stainable, in the later case an unstainable "symplasm" is produced.

According to the different formation and quality of the symplasm the development of new individual cells from this stage follows various lines. In all cases at first "regenerative units" become visible. These increase in size, turning into "regenerative bodies," which later, either by germinating or by stretching, become cells of normal shape. In some cases the regenerative bodies also return temporarily into the symplastic stage.

Besides the formation of the symplasm, another mode of interaction between the plasmatic substances in bacteria cells has been observed, consisting of the direct union of two or more individual cells. This "conjunction" seems to be of no less general occurrence than the process first mentioned. The physiological significance remains to be studied.

All bacteria multiply not only by fission but also by the formation of "gonidia"; these usually become first regenerative bodies, or occasionally exospores. Sometimes the gonidia grow directly to full-sized cells. They, too, can enter the symplastic stage. The gonidia are either liberated by partial or complete dissolution of the cell wall or they develop while still united with their mother cell. In the latter case the cell wall either remains intact or it is pierced by the growing gonidia, which become either buds or branches.

Some of the gonidia are filterable. They also produce new bacteria either directly or after having entered the symplastic stage.

The life cycle of each species of bacteria studied is composed of several subcycles showing wide morphological and physiological differences. They are connected with each other by the symplastic stage. Direct changes from one subcycle into another occur, but they are rather rare exceptions. The transformation of spore-free into spore-forming bacteria seems to be dependent on the conditions acting upon the symplasm and regenerative bodies.

The discovery of the full life cycles of bacteria may be helpful in many directions. Systematic bacteriology now can be established on a firm experimental basis. Physiological studies will win considerably in conformity and accuracy when connected with morphological investigations along these new lines. Several problems in general biology are brought under more promising aspects. Agricultural bacteriology and medical also will derive much benefit.

LITERATURE CITED

(1) ALMQUIST, Ernst.
1904. Neue Entwicklungsformen des Choleraspirills und der Typhusbakterie. *In* Centbl. Bakt. [etc.], Abt. 1, Orig., Bd. 37, Heft 1, p. 18–23.

(2) BASTIAN, H. C.
[1905]. The Nature and Origin of Living Matter. 344 p., 32 pl. Philadelphia, London.

(3) ———
1907. The Evolution of Life. 319 p., 12 pl. New York.

(4) COHN, Ferdinand.
1872. Untersuchungen über Bacterien. *In* Beitr. Biol. Pflanz., Bd. 1, Heft 2, p. 127–224, pl. 3.

(5) FOKKER, A. P.
1903. Versuch einer neuen Bakterienlehre. *In* Centbl. Bakt. [etc.], Abt. 1, Ref., Bd. 33, No. 1, p. 1–6.

(6) FÖRSTER, F.
1892. Ueber eine merkwürdige Erscheinung bei Chromatium Okenii Ehrbg. sp. *In* Centbl. Bakt. [etc.], Bd. 11, No. 9/10, p. 257–264, pl. 4 (col.)

(7) FUHRMANN, Franz.
1907. Entwicklungszyklen von Bakterien. *In* Verhandl. Gesell. Deutsch. Naturf. u. Ärtze, 78 Versamml. Stuttgart, 1906, T. 2, Hälfte 1, p. 278–279.

(8) ———
1913. Vorlesungen über technische Mykologie. 454 p., 140 fig. Jena.

(9) HEALY, D. J., and GOTT, E. J.
1916. A filterable organism isolated from the tissues of cholera hogs. *In* Jour. Infect. Diseases, v. 18, no. 1, p. 124–128, pl. 1.

(10) HENRI, Mme. Victor.
1914. Étude de l'action métabiotique des rayons ultraviolets. Production de formes de mutation de la bactéridie charbonneuse. *In* Compt. Rend. Acad. Sci. [Paris], t. 158, no. 14, p. 1032–1035, pl. 1.

(11) LEHMANN, K. B., and NEUMANN, R. O.
1912. Atlas und Grundriss der Bakteriologie . . . Aufl. 5, T. 1, Atlas, 79 pl. München.

(12) LEVY, Fritz.
1916. Über Copulationsvorgänge (?) bei Spirochaeta Obermeieri. *In* Arch. Protistenk., Bd. 36, Heft 3, p. 362–363, 1 fig.

(13) LÖHNIS, Felix.
1913. Laboratory Methods in Agricultural Bacteriology. Translated by William Stevenson and J. H. Smith . . . . 136 p., 39 fig., 3 pl. London.

(14) ——— and HANZAWA, J.
1914. Die Stellung von Azotobacter im System. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 42, No. 1/4, p. 1–8, 2 pl.

(15) MULVANIA, Maurice.
1915. Observations on Azotobacter. *In* Science, n. s. v. 42, no. 1083, p. 463–465.

(16) NEGRI, E. E. A. M. de.
1916. Untersuchungen zur Kenntnis der Corynebacterien, gleichzeitig ein neuer Beitrag zur Aetiologie des malignen Granuloms. *In* Folia Microbiol., Jahrg. 4, Heft 2, p. 119–187, pl. 9–16.

(17) PRAŻMOWSKI, Adam.
1880. Untersuchungen über die Entwickelungsgeschichte und Fermentwirkung einiger Bacterien-Arten. 59 p., 2 pl. Leipzig.

(18) PRAZMOWSKI, Adam.
1912. Azotobacter-Studien I. Morphologie und Cytologie. *In* Bul. Internat. Acad. Sci. Cracovie, Cl. Sci. Math. et Nat., S. B, No. 3, p. 87–174, pl. 7–9.

(19) SOROKIN, Nicolas.
1887. Eine neue Spirillum-Art. *In* Centbl. Bakt. [etc.], Bd. 1, No. 16, p. 465–466, illus.

(20) TRANSEAU, E. N.
1916. The periodicity of fresh-water algæ. *In* Amer. Jour. Bot., v. 3, no. 3, p. 121–133, 3 fig.

PLATE A

Magnification in all cases ×1,000. Preparates stained with cold aqueous fuchsin unless otherwise noted.

Fig. 1.—Azotobacter 11. Mannite-nitrate solution, 5 days old. Types A and La. Some cells in conjunction.

Fig. 2.—Azotobacter 21. Contact preparate from a colony on mannite agar, 4 days old. Types A, L. Most cells in conjunction.

Fig. 3.—Azotobacter 23. Contact preparate from a colony on mannite-agar, 4 days old. Types A, B, I, Kα, and many conjunct cells.

Fig. 4.—Azotobacter 13. Mannite-nitrate solution, 17 days old. Type Kλ.

Fig. 5.—Azotobacter 14. Mannite-nitrate solution, 5 days old. Type B forming I.

Fig. 6.—Azotobacter 8. Beef bouillon. Type B forming types I and J.

2

3

4

5

6

PLATE B

Magnification in all cases ×1,000. Preparates stained with cold aqueous fuchsin unless otherwise noted.

Fig. 7.—Azotobacter 21. Mannite-agar colony, 4 days old. Type C forming types D and I.

Fig. 8.—Azotobacter 22. Mannite-agar colony, 4 days old. Type C forming D also A in conjunction.

Fig. 9.—Azotobacter 11. From a filter paper strip in mannite-peptone solution, 16 days old. Types A and B forming D.

Fig. 10.—Azotobacter 3. Mannite-peptone solution, 24 days old. Types L and M forming D.

Fig. 11.—Azotobacter 11. Mannite-peptone solution, 16 days old. Type D (stained) resulting from type C.

Fig. 12.—Azotobacter 6. From condensation water of mannite-agar slant, 1 day old. Type D (unstained) containing regenerative units.

8

10

11 12

PLATE C

Magnification in all cases × 1,000. Preparates stained with cold aqueous fuchsin unless otherwise noted.

42721°—16——3

Fig. 13.—Azotobacter 24. Mannite-nitrate solution kept 5 days after having been heated 1 minute at 96° C. Types I and F developing from D. Some I germinating in conjunct stage and inclining to form spores.

Fig. 14.—Azotobacter 1. Mannite-nitrate solution, 10 days old. Types B, Kβ, E, and Fα developing from stained and unstained type D.

Fig. 15.—Azotobacter 15. From condensation water of a mannite-nitrate agar slant, 2 days old. Types Fα and Fβ developing from type D.

Fig. 16.—Azotobacter 17. Mannite-soil-extract agar, 2 months old. Types E, Fα, Kϕ, and G developing from type D.

Fig. 17.—Azotobacter 17. Mannite-nitrate agar, 10 days old. Preparate treated with hot aqueous fuchsin. Type G, partially dissolved; also Kϕ.

Fig. 18.—Azotobacter 7. Mannite-soil-extract solution, 14 days old. Type H forming D.

13

14

15

16

17

18

PLATE D

Magnification in all cases ×1,000. Preparates stained with cold aqueous fuchsin unless otherwise noted.

Fig. 19.—Azotobacter 2. Mannite-nitrate agar, 23 days old. Spores forming type D.

Fig. 20.—Azotobacter 2. Mannite-nitrate agar, 6 days old. Types L and F, endospores and exospores and dissolving of spores to type D.

Fig. 21.—Azotobacter 18. From a filter paper strip in mannite solution, 25 days old. Type L with gonidia, forming B (type Jλ).

Fig. 22.—Azotobacter 7. Mannite-soil-extract agar, 2 months old. Types E and F forming B.

Fig. 23.—Azotobacter 7. Mannite-soil-extract agar, 2 months old. Type B, formed by types E and F, germinating to type G.

Fig. 24.—Azotobacter 7. Mannite-soil-extract agar, 2 months old. Type Kγ.

19

20

21

22

23

24

PLATE E

Magnification in all cases ×1,000. Preparates stained with cold aqueous fuchsin unless otherwise noted.

Fig. 25.—*Bacillus subtilis* (No. 31). Beef agar, 2 days old. Types I and D formed by spores.

Fig. 26.—*Bacillus subtilis* (No. 31). Beef agar, 6 days old. Formation of type I.

Fig. 27.—*Bacillus subtilis* (No. 31). Beef agar, 8 days old. Type I forming H and stained D. Spores forming unstained type D.

Fig. 28.—Yellow bacillus (No. 41). Peptone-glycerin solution, 2 days old. Type I germinating from D, stretching to type L.

Fig. 29.—*Bacterium bulgaricum* (No. 49). Whey-yeast agar, 6 days old at 40° C. Types C, D, E, F, G, I, and K.

Fig. 30.—*Bacterium fluorescens* (No. 40). Ammonium-citrate-glycerin solution, 11 days old. Types D and H.

25

26

27

28

29

30

PLATE F

Magnification in all cases ×1,000. Preparates stained with cold aqueous fuchsin unless otherwise noted.

Fig. 31.—*Sarcina flava* (No. 43). Beef agar, 1 day old. Type I in conjunction and forming D.

Fig. 32.—*Streptococcus lactis* (No. 48). Peptone lactose solution, 5 days old. Type D, with regenerative units, forming type I.

Fig. 33.—*Streptococcus lactis* (No. 48). Milk, 3 days old. Types D and I in casein.

Fig. 34.—*Bacillus radicicola* (No. 39). Types D and I. Preparate made from a root nodule in 1908.

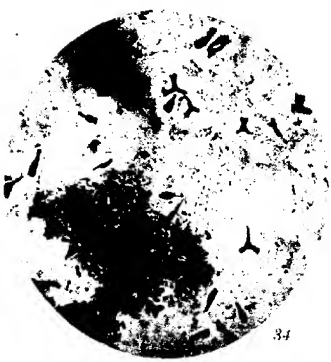
Fig. 35.—*Spirillum* sp. from Great Salt Lake (No. 46). Beef broth plus 3 per cent of sodium chlorid, 14 days old. Budding and branching forms; stained and unstained regenerative bodies. Some cells in conjunction.

Fig. 36.—*Spirillum* sp. from Great Salt Lake (No. 46). Beef broth plus 3 per cent of sodium chlorid, 14 days old. Type I germinating.

37

32

33



34

35

36

PLATE G

Magnification in all cases ×2,000. Preparates stained with cold aqueous fuchsin unless otherwise noted.

Fig. 37.—*Micrococcus candicans* from soil (No. 45). Ammonium-citrate-glycerin solution, 6 days old. Irregular, thick-walled type I.

Fig. 38.—*Micrococcus candicans* from milk (No. 44). Ammonium-citrate-glycerin solution, 2 days old. Irregular, thick-walled type I.

Fig. 39.—Yellow bacillus (No. 41). Beef agar, 1 day old. Budding gonidia, formation and germination of type I.

Fig. 40.—*Bacterium fluorescens* (No. 40). Ammonium-citrate-glycerin solution, 2 days old. Budding gonidia, formation and germination of type I.

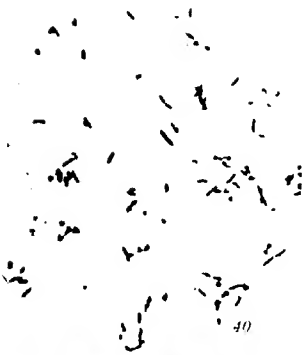
Fig. 41.—*Bacterium fluorescens* (No. 40). Beef agar, 4 days old. Filterable gonidia germinating.

Fig. 42.—*Bacterium fluorescens* (No. 40). Beef agar, 4 days old. Types D and F formed by filterable gonidia. Dark field.

37

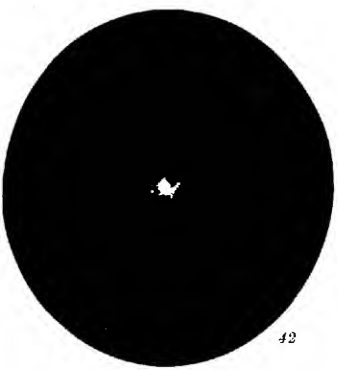
38

39



40

41



42

A RESPIRATION CALORIMETER, PARTLY AUTOMATIC, FOR THE STUDY OF METABOLIC ACTIVITY OF SMALL MAGNITUDE

By C. F. LANGWORTHY, *Chief*, and R. D. MILNER, *Assistant Chief, Office of Home Economics, States Relations Service*

INTRODUCTION

A respiration calorimeter of the type of that described in a previous number of the JOURNAL OF AGRICULTURAL RESEARCH,[1] which is employed in the laboratory of the Office of Home Economics of the Department of Agriculture for the study of the metabolism of matter and energy in the human organism, is easily adapted to inquiries of similar character with other organisms. An apparatus much smaller than the one referred to has been developed in the same laboratory for use in the study of gaseous exchange and energy transformations of small magnitude and has been employed in investigations on the ripening of fruits and the wintering of bees. In fundamental principle this small respiration calorimeter is similar to the larger one mentioned above in that it combines a closed-circuit respiration apparatus and a continuous-flow water calorimeter. However, it differs from it in construction, having been modified in ways which make for ease of operation and for greater accuracy. Important changes have also been made in details, particularly with reference to its calorimetric features, the use of special devices for controlling and recording temperatures rendering it quite largely automatic in this respect. Brief accounts of this apparatus and of experimental work with it have been published,[2] but the details of construction and operation are given for the first time in the present article. A general view of the small respiration calorimeter is shown in Plate XCII.

CONSTRUCTION OF THE RESPIRATION CHAMBER

The apparatus is devised so that chambers of different size or shape, constructed according to the varying needs of different investigations, can be substituted for each other. The chamber at present employed (Pl. XCIII) is 45 cm. square and 91 cm. deep, and has a total capacity of close to 185 liters. It was designed to accommodate a quantity of

[1] Langworthy, C. F., and Milner, R. D. An improved respiration calorimeter for use in experiments with man. *In* Jour. Agr. Research, v. 5, no. 8, p. 299–347, pl. 30–36. 1915.

[2] ——— An improved form of respiration calorimeter for the study of problems of vegetable physiology. *In* Orig. Com. 8th Internat. Cong. Appl. Chem., v. 18, sect. viiic, p. 229–236, 1 pl. [1912.]

——— A new respiration calorimeter for use in the study of problems of vegetable physiology. *In* U. S. Dept. Agr. Yearbook 1911, p. 491–504, pl. 65–67. 1912.

——— Some results obtained in studying ripening bananas with the respiration calorimeter. *In* U. S. Dept. Agr. Yearbook 1912, p. 293–308. 1913.

Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.

Vol. VI, No. 18
July 31, 1916
B—10

fruit sufficient for experimental purposes when stored in it under conditions approximating those of commercial practice, or otherwise, as desired. For example, a large bunch of bananas may be suspended in it from a removable cross of iron pipe the ends of which rest upon cleats fastened in the corners of the chamber near the top. Other cleats at different levels provide supports for shelves or for trays, baskets, or other containers in which the experimental material may be placed.

The walls of the chamber, which are of sheet copper 0.5 mm. thick, are attached to the inner side of a framework of hard-maple strips, experience having shown that using wood in place of metal lessens the possibility of error. The vertical strip in each corner of the frame is 3 cm. square, with the inner corner cut away on each side to a depth of 5 mm. to form a recess for the corner of the copper walls. At the top and bottom of the chamber, and midway between, the ends of cross strips 25 mm. square are joined to the posts so as to form a rigid supporting structure which is strong, though consisting of but little material. At the lower end each post extends 4 cm. below the bottom cross strip, to provide a leg for the structure. Elbows of stiff sheet copper, with one branch soldered to the outer surface of the copper wall and the other screwed to the framework, hold the copper firmly in place against the wooden frame.

At the top of the chamber is a close-fitting removable cover (Pl. XCIII) of sheet copper on a maple frame, with the metal projecting in a rim to hold the cover in place. The edge of the rim is bent down to fit into a groove in the flange formed by extending the copper side walls at the top to the outer edge of the maple frame. Wax melted into the groove seals the joint between the top and side walls when the cover is in place.

In the middle of the upper half of each of two opposite sides of the chamber is a framed opening 13 by 18 cm., forming a recess in which a pane of glass may be sealed (Pl. XCIII). These windows afford a view of the contents of the chamber and opportunity to watch the changes taking place. They may also be arranged so that either one may be opened during an experiment to remove a sample of the material under observation, if desired.

Another wall has a circular opening framed with a tube 9.5 cm. in diameter, in which is fitted a device called an "outlet" (see Pl. XCIII) which provides apertures for pipes conducting a ventilating current of air into and out of the chamber, for resistance thermometers providing passage for water entering and leaving the heat absorbers, for wires leading to electric-resistance thermometers inside the chamber, and for other purposes, as needed, all of which may be sealed in place. All openings into the chamber other than the windows are thus brought together in the one device, which is easily separated from the chamber so that the latter may be removed and another of different capacity substituted for it. During an experiment every joint in the chamber is air-tight.

As part of the arrangement described on page 716 for preventing the passage of heat through the walls, ceiling, and floor of the chamber, these surfaces are duplicated by sheet-copper top, bottom, and sides screwed to the outer edge of the wooden frame, the inner and the outer metal walls being thus separated by an air space 25 mm. across. There are openings in these walls for the windows and the "outlet" described above.

Surrounding the entire chamber, about 25 mm. from the outer metal wall, is a heat-insulating cover (Pl. XCIII) consisting of two layers of cork board 38 mm. thick, alternating with three layers of museum board 6 mm. thick, built up on wooden frames. The top, bottom, and side sections are built separately, and the several sections fit together with double-rabbeted joints, so that any one may be removed without regard to the others, or the entire cover may be instantly taken off. One section, as shown in Plate XCIII, is divided along the vertical median line, and all pipes and wires passing to the copper walls and to the "outlet" are brought out between the two halves of this section. The sections covering the two sides have openings to correspond with those in the walls of the calorimeter. The bottom section of the cover rests upon a substantial oak platform raised about 18 cm. from the floor of the laboratory.

DETERMINATION OF THE GASEOUS EXCHANGE

The respiration chamber in which the active material is confined is part of a closed air circuit through which a stream of air is constantly moving. The air which leaves the chamber is passed through purifying devices and returned again to the chamber. In the purifying devices the gaseous products resulting from the activity of the material in the chamber, which are carried out in the outgoing air, are absorbed. The purifying devices described below are those for the absorption of water vapor and carbon dioxid; but others could be substituted for these or connected with them if desired.

The quantities of water vapor and carbon dioxid carried from the chamber in a given period are shown by the changes in the weights of the absorbers during the period; and from these data, with due allowance for changes in the quantities of gases in the air of the chamber, the production of water vapor and carbon dioxid by the active material during the period is determined.

With a ventilation system of this type, as fast as any gas is removed from the air, other gas is introduced to maintain atmospheric pressure in the chamber. Usually oxygen is admitted, that being the gas consumed in respiration, as the term is commonly employed; but it is possible to vary the composition of the air at will, and if desired, to maintain an atmosphere of carbon dioxid or nitrogen or any other gas, which may be admitted to the system as oxygen is in the experiment as ordinarily conducted.

Oxygen to replace that consumed by the active material in the chamber is introduced into the air circuit from a reservoir of the gas. The quantity admitted is ascertained from the loss in weight of the container or by passing the gas through a meter. The amount admitted to the system and the change in the quantity of oxygen in the circulating air during a given period show the oxygen consumption of the material in the chamber.

A very light rubber bag on the end of a small copper tube extending from the chamber affords some variability in the capacity of the system, and by thus allowing for changes in the volume of gas present resulting from lack of uniformity in the rates of absorption of gas from the circulating air and the admission of other gas to it, or from changes in the temperature of the air within the chamber or in barometric pressure without, serves as an air-tension equalizer.

REMOVING WATER VAPOR AND CARBON DIOXID FROM THE AIR

The air withdrawn from the respiration chamber is forced first through sulphuric acid, which removes water vapor from it, and then through soda-lime, which removes carbon dioxid. The containers for the acid and the soda-lime, together with the air pump and the small electric motor by which it is actuated, are mounted on a stand with four shelves, called the absorber table (Pl. XCII). There are two parallel trains of absorbers on one shelf, one of which is in use while the units of the other are weighed and replenished.

The rotary air pump by which the circulation of air is maintained through the respiration chamber and the purifying devices has a capacity of approximately 100 c. c. per revolution, which is uniform for different rates of speed up to several hundred revolutions per minute. It is thus possible to vary the rate of ventilation of the chamber within a wide range simply by regulating the velocity of the pump, which is easily accomplished by means of a suitable rheostat to govern the speed of the motor which drives it. The rate of ventilation can be still further controlled, if desired, by means of a shunt in the air line between the inlet and outlet pipes of the air pump, with a valve to regulate the circulation through it. In the experiments for which the apparatus has thus far been used the former method has been sufficient, the pump being driven at a speed of 100 revolutions per minute, forcing 10 liters of air per minute through the system. With air ducts of brass pipe of 10-mm. bore the air flows in the circuit at very low pressure.

For absorbing water vapor from the circulating air, an acid bottle like that described for use with the large respiration calorimeter [1] but smaller in size has been found efficient. The bottle described by Williams,[2]

[1] Langworthy, C. F., and Milner, R. D. An improved respiration calorimeter for use in experiments with man. *In* Jour. Agr. Research, v. 5, no. 8, p. 306. 1915.

[2] Williams, H. B. Animal calorimetry. First paper. A small respiration calorimeter. *In* Jour. Biol. Chem., v. 12, no. 3, p. 323. 1912.

which is shown in Plate XCII, has also proved quite satisfactory. A charge of 500 c. c. of sulphuric acid in either of these bottles will continue for several hours to remove all water vapor from air passing through at any rate maintained in the experiments thus far conducted with the apparatus, even though in some cases water vapor in the air is near the saturation point. The bottle and the acid weigh less than 2 kgm., and by means of a sensitive balance of 10 kgm. capacity the change in weight during a given period is ascertained to an accuracy of 0.05 gm.

Carbon dioxid is removed from the air which leaves the acid bottle by soda-lime in a large-sized U-tube of special design (Pl. XCII). Each arm of the U consists of glass tubing 23 cm. long and 75 mm. in diameter, and the two are joined at the bottom by glass tubing of 15-mm. bore bent in a semicircle, leaving a narrow space between them. The upper end of each arm of the U-tube is closed by a ground-glass stopper from the top of which projects a glass tube of 10-mm. bore bent at right angles. The bottom of the stopper is closed except for an aperture of 10 mm., and in the space within the stopper is cotton wool to prevent particles of soda-lime from leaving the tube in the outgoing air.

A piece of fine-mesh brass wire gauze is put on the bottom of each large tube to keep the bent portion empty, and each arm is then filled to the stopper with soda-lime in particles of about the size of a dried pea, approximately 2 kgm. of soda-lime being required to fill both arms. This amount of material when fresh will commonly absorb at least 100 gm. of carbon dioxid before it needs attention, which is indicated by the color of the soda-lime. The gray-colored material, which is somewhat moist in the fresh condition, becomes white with use, owing to both loss of moisture and absorption of carbon dioxid. When the moisture is entirely gone the efficiency of the soda-lime is low; but by passing moist air through the tube it may be restored to such an extent that the soda-lime may be used for at least one more period.

To catch the moisture given up by the soda-lime to the dry air coming from the first water absorber, the air leaving the U-tube is passed through another bottle of acid. Both the acid bottle and the U-tube, for which there is easily room on the pan of the balance by which the gain in weight of the absorbers is determined, are weighed together to find the quantity of carbon dioxid removed from the circulation of air. Their total weight is less than 5 kgm., and their change in weight is ascertained to an accuracy of 0.05 gm.

The air leaving the second acid bottle is passed through a trap of cotton wool (Pl. XCIII) to catch any spray that might be carried from the sulphuric acid by the moving air. The quantity of acid that leaves the absorber is so small that the trap need not be weighed.

SUPPLYING OXYGEN TO THE AIR

The oxygen supplied to the chamber to replace that absorbed by the active material is obtained from a cylinder which contains the gas under pressure. It is admitted at such a rate that the apparent volume of gas in the chamber as indicated by the tension equalizer is relatively constant. The regulation may be by hand; or the tension equalizer may be arranged to cause a valve in the oxygen feed line to be opened or closed as the volume of gas in the system diminishes or increases. The small cylinder with the gas-pressure-reducing valve attached weighs less than 10 kgm., and changes in the weight of it may be ascertained to an accuracy of 0.05 gm., which means that the actual volume of the gas admitted is known to within 50 c. c. With regularity in the rate of admission of oxygen, other methods of determining the quantity, as by means of an accurate meter carefully calibrated, or by the filling and emptying of a calibrated spirometer, are suitable. In the latter case a sensitive spirometer could serve also as a tension equalizer.

CHANGES IN THE COMPOSITION OF THE RESIDUAL AIR

At the beginning and the end of each experimental period a portion of the air leaving the rotary pump is shunted through a train of small absorbing devices [1] and then through an accurate meter, which stands on the top shelf of the absorber table. The air leaving the meter is restored to that in the main line returning to the chamber. The weight of each small absorber, which is less than 100 gm., is ascertained to an accuracy of 0.1 mgm. The quantities of water vapor and carbon dioxid in the measured sample of air, as shown by the increase in the weights of the small absorbers, represent very accurately those of the atmosphere of the chamber. With such material as ripening fruit in the chamber, any change in the composition of the atmosphere occurs so slowly that it has no appreciable effect on the air of the chamber during the period of taking the sample. A fan to stir the air is unnecessary, the total volume being small when the quantity of active material used for experimental purposes is inclosed in the chamber. The air is withdrawn from the chamber through a pipe terminating at the floor in a cross with both ends open, while air is returned to the chamber through a pipe opening near the ceiling. The circulating air thus traverses the full depth of the chamber. At the usual rate of ventilation the total volume of air in the system completes the circulation several times per hour.

When a sample of air is desired for the determination of the proportion of oxygen present, it is usually taken from that returning from the absorbers to the chamber, no oxygen being admitted from the cylinder to the system at the time. Ordinarily this determination is not necessary, since by properly accounting for the different products removed from and admitted to the ventilating system, the quantity of oxygen consumed from the atmosphere may be computed.

[1] Langworthy, C. F., and Milner, R. D. Op. cit., p. 311.

In the computation of the quantities of water vapor, carbon dioxid, and oxygen in the atmosphere of the chamber the actual volume of air in the chamber must be known, and this depends upon the capacity of the chamber under standard conditions of temperature (0°C.) and of barometric pressure (760 mm. of mercury) and the actual temperature and pressure of the air at the time the samples were taken. The barometric pressure of the air of the chamber, because of the tension equalizer, is always the same as that of the laboratory, which is determined to an accuracy of 0.01 mm. by means of a standardized barometer. The temperature of the air of the chamber is measured by means of an electric-resistance thermometer with the sensitive portion in the chamber and a temperature indicator outside. Either of two thermometers is available, one consisting of a single unit and the other of three units in series, which are modifications of the type of thermometer developed by Dickinson and Müller.[1] They are very sensitive and follow temperature changes rapidly. The single unit consists of a coil of nickel wire having a resistance of about 20 ohms at 20° C., wound on a very thin strip of mica, placed between two similar strips, and inclosed in a flat case of thin copper pressed firmly against the mica. The portion of the case which incloses the coil is about 15 cm. in length, 13 mm. in width, and less than 1.5 mm. in thickness. The case terminates at the top in a short tube, through which the leads are extended to the resistance wire, being sealed in the tube with a hard wax to exclude moisture from the interior of the case. Each of the three units in series is constructed like the one just described, except that it has only one-third the total amount of resistance wire; hence, the unit is shorter, the other dimensions being the same.

The leads from the resistance thermometer coils pass through the "outlet" mentioned on page 704 and extend to a multiple-switch (Pl. XCIV), by which either the single or the triple thermometer may be connected with the temperature indicator, which does not appear in any of the views shown. The latter device consists of a Wheatstone bridge having a slide wire by which the bridge circuit may be kept in balance with the thermometer coils at any temperature between 0° and 50° C. The readings of the bridge scale, when translated into temperature by means of a calibration curve, show changes to 0.1°. The effect of the resistance of the thermometer leads and of their change in resistance, due to change in temperature, is neutralized by compensating leads from the opposite side of the bridge, so that the measurements by means of the bridge are of a high order of accuracy; although, because of the small volume of air in the chamber, absolute accuracy of these determinations is of less significance than in experiments with the larger respiration apparatus.[2]

[1] Dickinson, H. C., and Mueller, E. F. New calorimetric resistance thermometers. *In* U. S. Dept. Com., Bur. Standards Bul., v. 9, no. 4, p. 483-492, 2 fig. 1913.
[2] Langworthy, C. F., and Milner, R. D. Op. cit., p. 312.

DETERMINATION OF THE QUANTITY OF HEAT PRODUCED

The amount of heat resulting from the activity of the material in the respiration chamber is ascertained from determinations of (1) the quantity of latent heat in the water vapor of the outgoing water; (2) the quantity of sensible heat absorbed and carried away by water flowing in a coil of pipe in the chamber; and (3) the quantity of heat involved in changes in the temperature of the active material and of other objects in the chamber and also of the walls of the chamber. The gain or loss of sensible heat through the walls or in the ventilating current of air is prevented.

LATENT HEAT

The quantity of water vapor carried from the chamber is determined from the gain in weight of the first sulphuric-acid bottle in the absorber train, as explained on page 706. If this quantity is multiplied by the factor 0.586, the product will be the number of Calories of latent heat at 20° C. carried from the chamber in the water vapor of the outgoing air.[1]

SENSIBLE HEAT

Sensible heat emanating from the active material is removed from the chamber by a current of water flowing in a heat absorber, and the amount of heat thus removed in a given period is determined from the weight of water that flows through the absorber during the period and its temperature increase, with due allowance for the specific heat of the water at the mean temperature of the flow as compared with that at the temperature taken as standard. By controlling the rate at which water flows through the heat absorber, or the temperature at which it enters the absorber, the removal of heat is made to accord with its production, so that the temperature of the air of the chamber is kept as closely as possible to that which it is desired to maintain.

The heat absorber consists of 15 m. of copper tubing of 3-mm. bore in a double coil soldered to the upper and under surfaces of a piece of sheet metal 38 cm. square, with a double loop of pipe about 80 cm. long extending downward from each edge of the sheet. The absorber is removable and is slipped into position after the material under observation has been packed in the chamber. When in position, it is suspended with the sheet metal parallel to the ceiling of the chamber and about 25 mm. below it, with the double loops extending down the sides of the chamber and about 25 mm. from them.

REGULATING THE RATE OF THE WATER FLOW

The water for the heat absorber flows from a constant level reservoir on a shelf above the calorimeter chamber, which is supplied from a tank on the lower shelf of the absorber table. The water that leaves the absorber is returned to the tank, from which it is raised again to the

[1] Langworthy, C. F., and Milner, R. D. Op. cit., p. 315.

reservoir by a small gear pump driven by the motor that actuates the air pump. The overflow from the reservoir also returns to the tank. The same water is used continuously in this manner to eliminate the difficulty resulting when water directly from the city main is passed through the system, owing to the presence of air dissolved in the water. If the temperature of the water is raised, the air escapes and collects in bubbles in the pipe and forms temporary obstructions that cause irregularity in the rate of flow of water through the absorber. With well-filtered water in the system a rate of flow as low as 5 liters per hour has been maintained with such uniformity that it would be sufficient to collect the water leaving the heat absorbers at intervals instead of continuously. Slight changes in the rate may be effected by the adjustment of a glass rod, with a long tapering end which passes through a constricted orifice in one end of a glass T-tube in the water line.

That the air of the chamber may be kept at any desired temperature, water is usually allowed to flow through the heat absorber at a constant rate and the temperature of the ingoing water is varied in accordance with the quantity of heat to be absorbed. To bring this temperature under control, the water is first cooled below that at which it is to be used, and then heated to the desired temperature. In these circumstances regulation of temperature is accomplished simply by variation in the amount of heating, which is easily controlled automatically.

REGULATING THE TEMPERATURE OF THE WATER FLOW

The water flowing from the reservoir to the heat absorber passes first through a pipe immersed in cold water to chill it, and then into a device called the preheater (Pl. XCIV) in which, by the conversion of electric current into heat in resistance coils inclosed in the water channel, the temperature of the water may be raised several degrees. The total heating effect of the device will increase the temperature of the water nearly 6° when the rate of flow is not over 500 c. c. per minute, and the heat may be added in small quantity. By this means the temperature of the water is raised near to that at which it is to enter the absorber. From this device, which is adjusted by hand, the water passes to the final heater (Pl. XCIV), which has a smaller capacity than the preheater, but is automatic and regulates the temperature within very narrow limits. The device is similar in some respects to that employed with the large calorimeter,[1] while in others it has been considerably simplified and improved.

The temperature of the water is raised or lowered by increasing or decreasing the electric current flowing in a coil of resistance wire immersed in the water. This is accomplished by adjusting the position of the sliding contact on a rheostat wound with resistance wire of graduated

[1] Langworthy, C. F., and Milner, R. D. Op. cit., p. 319.

cross section in series with the heating coil. The contact is moved by a motor-driven mechanism, the movement being governed by the deflection of the pointer of a sensitive galvanometer incorporated in the mechanism. The terminals of the galvanometer are connected with a special Wheatstone bridge (the temperature indicator shown at *F* in Pl. XCIV), one arm of which is a resistance thermometer installed in the upper half of the water channel in the final heater, so that it is submerged in the water flowing past the heating coil in the lower half of the channel. The slide wire of the bridge is calibrated to cover a range of temperature from 0° to 35° C., and the scale of the bridge is graduated to 0.1°. If the temperature of the stream of water in which the thermometer is immersed differs 0.05° from that at which the pointer of the temperature indicator is set, the needle of the galvanometer is deflected, the direction in which it swings depending upon whether the temperature of the water is too high or too low, and the amplitude of the swing depending upon the amount of the difference in temperature. The effect of any deflection is a shift in the position of the contact on the rheostat, which alters the current in the heating coil and thereby varies its heating effect. This continues until the water is brought to the desired temperature. The extent of change in the temperature of the water at any single shift of the contact on the rheostat varies according to the magnitude of the deflection of the pointer, from one of an extremely small fraction of a degree to one of about 0.1°. The cam shaft by which the contact is shifted rotates in less than 3 seconds, so that alteration in the heating current may occur every 3 seconds if necessary. Thus, the temperature of the water may be changed very quickly; or, in other words, any variation in its temperature from that desired may be rapidly corrected.

From the final heater the water flows to the bottom of a bottle of about 3 liters' capacity (Pl. XCIV, *B*), filled with small pieces of pumice, from the top of which it flows to the heat absorber at a very steady temperature.

It has been stated on page 710 that one purpose of controlling the temperature of the ingoing water is to keep the temperature of the air within the chamber as constant as possible. The operator counteracts any tendency towards change in the temperature of the air by changing the setting on the indicator for the temperature of the water entering the heat absorber. By a slight modification in arrangement this could be made automatic. The resistance thermometer for the temperature of the air in the chamber could be connected with the temperature indicator in place of the thermometer in the final heater, so that whenever the temperature of the air varied from that set on the indicator the device for regulating the temperature of the water entering the heat absorber would be changed in such a manner as to correct it.

MEASURING THE TEMPERATURE INCREASE

In the passage of the water through the heat absorber its temperature will increase according to its rate of flow and the quantity and activity of the material in the chamber. The accuracy with which the amount of heat carried from the chamber in the water current is measured depends upon that with which the temperature increase is determined. This is accomplished by means of electric-resistance thermometers and an automatic temperature recorder (Pl. XCV), in some respects similar to and in others different from that employed in connection with the large respiration calorimeter.[1]

In construction and characteristics the resistance thermometers are identical with those in the large calorimeter. They consist of two coils of platinum wire of equal resistance, which is about 25.5 ohms at 20° C., and have exactly the same coefficient of change in resistance with change in temperature, the resistance change of each being 0.1 ohm per degree. In each the resistance coil is encased in such a way that it is brought into intimate thermal contact with the flowing water and responds instantly and accurately to any change in its temperature. The water channels in which the resistance coils are installed are fitted into openings in the outlet described on page 704, to provide passage through the walls of the chamber for the ingoing and outgoing water, so that one coil acquires the temperature of the water just entering and the other that of the water just leaving the chamber.

The thermometers comprise two arms of a special Wheatstone bridge on opposite sides of a slide wire by which the bridge may be balanced for any inequality in the resistance of the two coils between 0.001 ohm and 0.2 ohm, resulting, respectively, from temperature differences of 0.01° and 2° between the ingoing and the outgoing water. The wire is calibrated so that temperature differences may be read directly from the scale. The total range of the instrument may be extended to indicate a difference as large as 5°. By means of resistance coils that may be connected in series with the slide wire as needed, the position of the balancing contact on the lower end of the wire may be made equivalent to a difference of 1°, 2°, or 3° between the thermometer coils, and the upper end 2° higher in each case.

The slide wire is incorporated in a mechanism which automatically balances the bridge for inequalities of resistance in the thermometers, and at the same time makes a graphic record of the balancing operations in terms of temperature difference and of time. The wire is mounted on the edge of a disk which is rotated to balance the bridge, while the balancing contact point remains fixed. The rotation of the disk, which is due to the action of one or the other of two cams on a shaft driven by

[1] Langworthy, C. F., and Milner, R. D. Op. cit., p. 326

a small electric motor, is governed by the deflection of the pointer of a very sensitive galvanometer, which is also incorporated in the mechanism, with its terminals connected with the Wheatstone bridge. When the bridge is in balance, the pointer remains at the zero position, and the slide wire is not moved; but any variation in the temperature of the water in either thermometer results in a change of resistance in the thermometer coils that upsets the balance of the bridge, the pointer swings to one side of the zero position or the other, according to the relation between the resistances of the opposite branches of the bridge, and the disk is turned in that direction in which the slide wire should be moved to restore the balance of the bridge. The amount of change in the position of the contact point on the slide wire is proportional to the magnitude of the swing of the pointer, which depends on the temperature difference in the thermometer coils. A difference of 0.005°, or even less, will upset the balance of the bridge sufficiently to cause a swing of the galvanometer pointer that will result in a movement of the disk. However large the temperature difference might be at any given instant, because of certain mechanical details connected with the mechanism for rotating the disk, stops are provided to limit the swing of the pointer either side of zero to that which would result from an inequality of resistance due to a difference of nearly 0.2° in the thermometer coils; but the cam shaft rotates every 5 seconds, and the disk may be moved that often if necessary; hence, the mechanism will keep the bridge balanced for inequalities resulting from any change of temperature difference in the ingoing and outgoing water up to 2° per minute.

The shaft on which the disk rotates also causes a pen to draw a line on ruled paper to show the direction and the distance that the slide wire had to be moved to balance the bridge. In the width of paper corresponding to the length of the slide wire that is equal to a difference of 2° there are 100 lines. The distance from line to line, which represents a temperature of 0.02°, is 2.5 mm. Hence, the temperature difference indicated by the position of the pen at any instant may be easily read to 0.01°.

The current in the bridge circuit is not sufficient to cause an increase in the temperature of the thermometers that will produce a movement of the pen even when the water is flowing through the thermometer at a rate much lower than the lowest that would be used with the apparatus.

A differential thermoelement is installed in the resistance thermometers so that the temperature difference of the water in the bulbs may be determined by means of a potentiometer as a check upon the measurement by the recorder. The Wheatstone bridge is provided with duplicate parts, which, by substitution, serve as means of checking the accuracy and constancy of the resistances of the bridge.

CHANGE IN TEMPERATURE OF OBJECTS IN THE CHAMBER

Any change in the temperature of the walls of the chamber, or of the material confined within them, involves a quantity of heat for which allowance should be made in computing that produced in the chamber. For example, if the walls of the chamber are warmer at the end than at the beginning of the experiment, they have absorbed some of the heat that was produced in the chamber; while if they are cooler at the end of the experiment, some of their heat has been added to that in the chamber. The quantity of heat for which allowance must be made is computed from the change in the temperature of the walls and their hydrothermal equivalent—that is, the amount of heat involved per degree of temperature change in the walls.

The change in temperature is determined by electric-resistance thermometers devised for this apparatus. The resistance wire is wound in a flat coil about 5 cm. in diameter, which is firmly attached to one surface of a disk of stiff brass 55 mm. in diameter and 1.5 mm. thick. Through a hole in its center the disk is slipped over a short brass bolt projecting from the surface of the copper wall, so that by screwing a nut down on the bolt the disk may be clamped tightly against the wall, with the resistance wire between them. Between the wall and the wire are two or three layers of tinfoil to provide thermal contact in case of irregularity in the copper. The whole thermometer comprises 10 such coils, one for the top, one for the bottom, and one for the upper half and one for the lower half of each side. Each coil has a resistance of 45 ohms, but the 10 coils are connected in a series parallel arrangement to form a unit having a resistance of about 18 ohms at 20° C. The leads from this unit connect with the special switch and the Wheatstone bridge mentioned on page 709. The galvanometer will indicate a lack of balance due to a change of 0.05° in the temperature of the walls.

The most satisfactory data obtained in determining this factor indicate that for a change of 1° in the temperature of the walls the correction in the quantity of heat measured by the calorimeter would not exceed 1.5 Calories.

The correction involved in the change in temperature of the active material in the chamber is computed from the weight and specific heat of the material, and the temperature change as measured by an electric-resistance thermometer. One or the other of the two thermometers mentioned on page 709 is put into the mass of active material in such manner as to be in intimate contact with it. Tests with ripening fruit have shown that thermometers used in this manner indicate temperature change at least as accurately as a thermometer thrust into the flesh of one of the fruits.

PREVENTING GAIN OR LOSS OF HEAT IN THE CHAMBER

Part of the arrangement for preventing increase or decrease in the amount of the heat in the chamber by the passage of heat through the metal walls consists in duplicating the side walls, ceiling, and floor of the chamber by parallel surfaces of sheet metal attached to the outside of the wooden frame, as explained on page 705. For convenience, the metal walls which actually confine the chamber—in this connection all six surfaces being considered walls—are designated the inner walls, while the corresponding surfaces on the outside of the frame are called the outer walls. If the temperature of the outer wall is regulated so as to keep it always like that of the inner wall, neither will transmit excess of heat to the other, and consequently there will be no gain or loss of heat through the walls.

The temperature of the outer wall is regulated by heating and cooling the air in the narrow space between the wall and the heat-insulating cover described on page 705. The air is cooled by chilled water flowing in a small-bore copper tube in the space and it is heated by the conversion of electric energy into heat in a resistance wire parallel with the pipe. The wire and the pipe for controlling the temperature of the side walls are shown in Plate XCIII. The chilled water flows through the pipe continuously at such a rate that the air in the space will be too cool when the heating effect of the electric current in the resistance wire is near its minimum, and the current in the resistance wire is regulated until the air is heated to the desired temperature. In these circumstances the temperature of the air may be raised or lowered simply by varying the current in the resistance wire, which is accomplished by adjusting a rheostat in series with the wire.

The rheostat is adjusted automatically by a motor-driven mechanism (Pl. XCV). The resistances of the rheostat are arranged in a circle about a shaft by which the contact point is shifted to vary the amount of resistance in series with the heating wire. The direction in which the shaft will turn depends upon the deflection of the pointer of a galvanometer mounted in the shifting mechanism, with its terminals connected to a Wheatstone bridge, two arms of which consist of electric resistance thermometers attached to the inner and the outer metal walls of the chamber. The coils of these thermometers are identical in construction with those described on page 715 and are similarly attached to the outer surface of the inner wall and the inner surface of the outer wall, the disks on the inner wall forming one arm and those on the outer wall the opposite arm of the bridge. The two units are identical in resistance at the same temperature, and with the galvanometer employed they form a very sensitive differential thermometer that is influenced by small changes in the thermal condition of the walls. If the temperature of the outer wall differs by as much as 0.01° from that of the inner wall, the

resistances of the two parts of the thermometer will differ accordingly, and the pointer of the galvanometer will be deflected, the direction of deflection depending upon whether the outer wall is warmer or cooler than the inner, and the contact point of the rheostat will be shifted so as to increase or decrease the heating of the outer wall and bring it again into thermal equilibrium with the inner wall.

Thermal equilibrium is maintained in the walls by sections rather than as a whole. The resistance coils on the inner and outer metal walls are grouped so that the top, the sides, and the bottom of the chamber each has its own differential thermometer; and provision is likewise made for heating and cooling each section independently, so that thermal conditions in each one may be regulated regardless of those in the others. Furthermore, in order that there shall be no excess of sensible heat carried into or out of the chamber in the ventilating current of air, the temperature of the air entering the chamber is regulated to accord with that of the air leaving. The units of a differential resistance thermometer are inclosed in the pipes carrying the ingoing and outgoing air through the walls of the chamber. Just before the pipe for incoming air reaches the calorimeter a short section of it is inclosed in an electric heating device to warm the air, while inside the same section of pipe is a small copper tube conducting chilled water to cool the air. As in the control of the temperature of the walls, the water is kept running continuously and the temperature of the air is regulated by varying the electric current in the heater surrounding the air pipe. The four rheostats controlling the currents for heating the top, sides, and bottom outer walls of the chamber, and the ingoing air are adjusted by the same mechanism (Pl. XCV), which operates them successively, any changes that are needed in a given rheostat being made once every four minutes.

The widest difference between the respiration calorimeter described in the present article and the larger one previously described in this journal[1] is in the method of preventing gain or loss of heat in the chamber. The devices described in the paragraphs above render this apparatus quite largely automatic in its operations as a calorimeter, whereas the other calorimeter is controlled mainly by hand.

By means of a switch, also operated by the mechanism, the galvanometer which governs the action of the regulating mechanism upon the rheostats is connected successively across each of the four Wheatstone bridges of which the differential thermometers are integral parts, each pair of thermometers being combined with its own ratio coils to form a bridge. These four sets of coils, are mounted in the same case (Pl. XCV) in such a manner that the permanence of resistance of each may be easily tested. The coils in each pair may be transposed by changing the position of two plugs, whereupon the galvanometer deflection will alter

[1] Langworthy, C. F., and Milner, R. D. Op. cit., p. 326.

if the coils differ in resistance. Moreover, the ratio coils of one bridge may be combined with those of either of the other three to form a test bridge, all four arms of which should have the same resistance. With a very sensitive galvanometer across the bridge thus formed, any inequality in the coils would be detected. It is assumed that if there is no deflection the coils have not changed in resistance, since it is hardly probable that all the coils would have changed equally. If any change should be detected, by varying the combinations it would be possible to determine which pair of coils was at fault. By shifting the point of contact of the battery lead on a short wire joining the two coils, equality of resistance may be restored when the changes are slight. No tests of this character have thus far indicated any need for change. Each bridge is also provided with a small variable shunt across a small resistance in series with one of the two differential thermometers to compensate for small inequalities in their resistances when at the same temperature.

TESTS OF THE ACCURACY OF THE RESPIRATION CALORIMETER

The accuracy with which it is possible under given conditions to measure the factors studied by means of the respiration calorimeter is shown by a comparison of the determined amounts of oxygen consumed and of carbon dioxid, water vapor, and heat produced upon combustion of ethyl hydroxid in the chamber with those which should result from the combustion as calculated from the quantity of alcohol burned and the percentage of ethyl hydroxid in it.

A burner inside the chamber is connected with a small-bore copper tube that passes through the "outlet" in the wall of the chamber. To the exterior end of this tube is attached a glass U-tube with one long arm into which alcohol for the burner is fed by dropping from a supply bottle which may be weighed at intervals to determine the quantity burned. To test the apparatus under conditions equivalent to those of experiments in which it is used, the alcohol must be burned at a very slow rate. Some difficulties were experienced at first in attempts to burn as little as 1 gm. per hour with complete combustion of the alcohol at a constant rate and with inappreciable loss by evaporation from the long arm of the U-tube. These were due in part to the fact that the opening in the "outlet" through which the alcohol tube passed is considerably above the level at which it is desired to have the combustion take place in the chamber. As a result of this condition, in all the tests thus far made the level at which the alcohol was maintained in the vertical tube was above that at which it was burned, attempts to feed the burner by siphon having proved unsuccessful. It was necessary to devise a burner which would overcome the effect of the pressure of the alcohol in the feed tube upon the rate of flow.

Burners of small-bore glass tubing of various diameters and with wicks of cotton, of glass wool, and of granited asbestos, packed so as to allow

the alcohol to escape at the desired rate, were tried, but most of them were worthless because after combustion had continued a short time the flow of alcohol would begin to diminish and finally would be stopped entirely by material deposited in the top of the wick. This would occur even when the upper part of the wick was removed so that there was clear alcohol to a depth of 3 mm. or more below the flame. The phenomenon appeared to be associated with incomplete combustion of the alcohol, because whenever it occurred evidence that combustion was not complete could be found in the air of the chamber. That the material deposited in the wick was not in solution or in suspension in the alcohol was indicated by the fact that a sample of 100 gm. from the supply bottle when evaporated left a residue less than 0.1 mgm.

Some successful results were obtained with a burner of very thick wall and a bore of approximately 1 mm., with glass wool for a wick. When the glass wool was sufficiently tamped some pressure was necessary to force alcohol through it at the desired rate. The chief objection to this burner was the tendency of the thick tube to crack when the alcohol was lighted. Alcohol was fed from the supply bottle by dropping at such a rate that the level of the alcohol would remain at a mark on the long arm of the U-tube indicating the height which had been found by trial to produce sufficient pressure to keep the alcohol burning at the desired rate. This U-tube was of small bore to reduce the surface from which evaporation could take place, and the open end of the tube was nearly closed by the constricted nozzle of the tube from the supply bottle, leaving only a small space through which vapor could escape.

The results obtained with a burner consisting of two concentric small tubes and a wick of asbestos tape filling the narrow annular space between them were also quite satisfactory. No products of incomplete combustion were found in the air of the chamber when alcohol was burned in either of these burners at a rate even lower than 1 gm. per hour. The data in Table I show the results of two representative tests.

TABLE I.—*Data obtained in the combustion of alcohol in the respiration calorimeter*

Date.	Duration.	Weight of alcohol burned.	Water.		Carbon dioxid.		Oxygen.		Heat.		Respiratory quotient, $CO_2:O_2$.
			Found.	Required.	Found.	Required.	Found.	Required.	Found.	Required.	
1914.	*Hours.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Cal.*	*Cal.*	
Jan. 21.....	5	9.20	9.7	10.7	15.7	16.2	17.4	17.7	61.2	60.4	0.655
	5	9.22	10.1	10.7	16.0	16.2	17.3	17.7	61.0	60.5	.653
Jan. 27.....	10	18.42	19.8	21.4	31.7	32.4	34.7	35.4	122.2	120.9	.663
	11.75	10.34	11.8	11.9	16.7	17.2	18.4	18.7	.....	.....	.660

In the test of January 21 the alcohol was burned at a rate averaging slightly more than 1.8 gm. per hour. Almost exactly the same total quantity was burned in each of the two consecutive 5-hour periods com-

prising the test, and the determinations of carbon dioxid, oxygen, and heat in one period agree quite closely with those in the other, while the discrepancies between the quantities found and those required are for the most part small. The ratio of the volume of carbon dioxid produced to that of oxygen consumed in the test was 0.663, whereas the theoretical respiratory quotient for the combustion of alcohol is 0.667.

The only test in which less than 1 gm. of alcohol per hour was burned for any considerable period was that on January 27, which continued nearly 12 hours, at a rate of combustion averaging only 0.88 gm. per hour. There was a close agreement between the quantities computed and those determined in the measurement of gaseous exchange in this test also, but the heat production was not determined. These results are quite typical of all those obtained in tests of this character. In none of them have there been wider discrepancies than these shown between the measured and calculated quantities, the reasons for which were not ascertained and which could not have been avoided.

The accuracy with which heat generated in the calorimeter chamber can be determined is tested also by converting a known amount of electrical energy into heat in a resistance coil suspended in the chamber and measuring the heat with the calorimeter. In a test made on February 3, 1914, a current of 0.087 ampere was passed through a resistance coil of 1,680 ohms at an average pressure of 146.5 volts, generating

10.97 Calories of heat per hour according to the formula $\frac{I^2R}{4.183}$ = small

Calories per second at 20° C. The quantity of heat measured by the calorimeter was 11.04 Calories in the first hour and 11.08 Calories in the second hour of the 2-hour test. During the second hour the increase in the temperature of the water that flowed through the heat absorber in the chamber was measured by a potentiometer and the differential thermoelement installed in the resistance thermometers (see p. 713) as a check on the measurement by the thermometers themselves. The average temperature difference was 1.42° as indicated by the resistance thermometers and recorder and 1.40° by the thermoelement and potentiometer. The discrepancy between the computed and the measured amounts of heat in the second period of this test is wider than that in any other electric test with this respiration calorimeter. The closest agreement was that obtained in a test which continued only 1 hour, on November 2, 1912, in which the amount of heat computed to have been generated in the chamber was 7.54 Calories and that measured by the calorimeter was 7.56 Calories.

Both the electric and the alcohol tests indicate that measurements can be made with this respiration calorimeter to a high degree of accuracy.

PLATE XCII

General view of the respiration calorimeter

A, Chamber inclosed in heat-insulating cover. *B*, Tension equalizer to maintain atmospheric pressure in the air of the chamber. *C*, Absorber table. *D*, Rotary pump to maintain air circulation. *E*, Motor to drive pump. *F*, Bottles containing sulphuric acid to remove water vapor from circulating air. *G*, Large U-tube, containing soda-lime to remove carbon dioxid from the air. *H*, Bottle containing sulphuric acid to catch the water vapor from the soda-lime. *I*, Bottle containing cotton to catch sulphuric acid vapor. *J*, Small absorbers for determining water vapor and carbon dioxid in residual air. *K*, Meter to measure the sample of residual air. *L*, Reservoir to maintain a constant pressure of water in the heat absorber in the chamber. *M*, Tank to catch water flowing from the heat absorber. *N*, Pump to raise water from the tank to the reservoir. *O*, Devices for automatically controlling and recording temperatures.


L
O
K
C
G
B
A
M
D
N
E

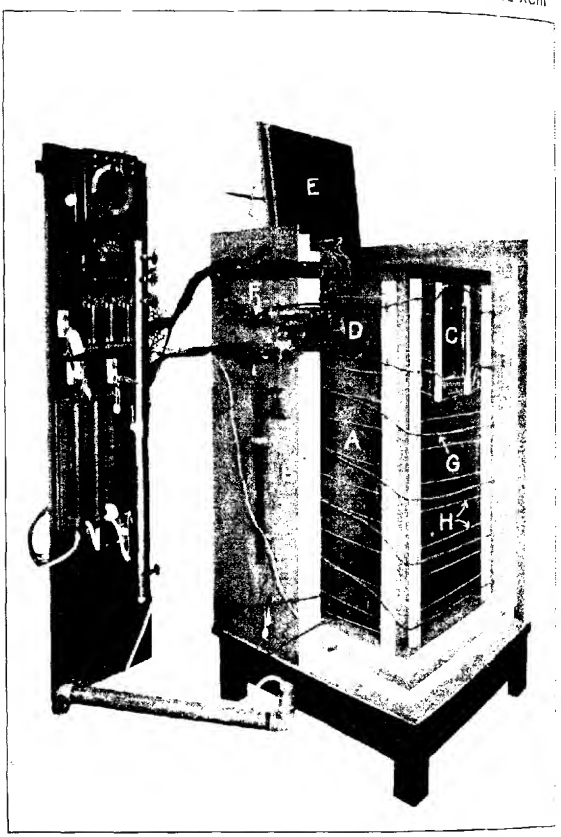

E
L
F
D
C
A
G
H

PLATE XCIII

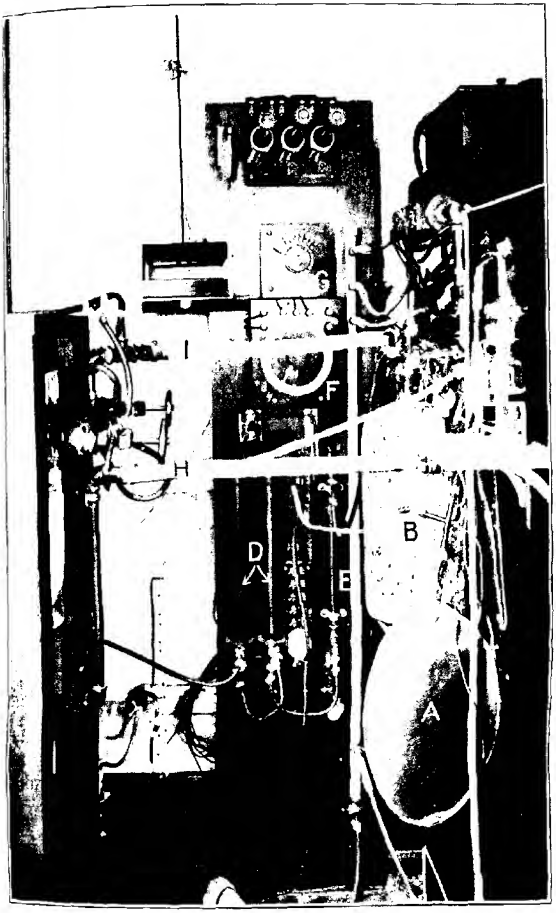
Chamber with part of outer covering removed

A, Double metal wall chamber. *B*, Heat-insulating outer cover. *C*, Window to chamber. *D*, Outlet providing passage for pipes, wires, etc., through the walls of the chamber. The exterior ends of the resistance thermometers for ingoing and outgoing water are seen projecting from the outlet. *E*, Removable top of chamber. *F*, Device for heating the air entering the respiration chamber. *G*, Small pipe carrying water for cooling the outer metal wall of the chamber. *H*, Electric-resistance wire carrying current for heating the outer wall.

PLATE XCIV

Apparatus connected with the respiration calorimeter

A, Tension equalizer. *B*, Mixing bottle for equalizing the temperature of water entering the heat absorber. *C*, Device for heating air entering the respiration chamber. *D*, Preheater, and *E*, final heater, for raising the temperature of water entering the heat absorbers. There is an electric-heating coil in the lower half and an electric-resistance thermometer in the upper half of the final heater. *F*, Temperature indicator comprising part of the apparatus for controlling the temperature of the water entering the heat absorber. This device is connected with the resistance thermometer in the final heater and with the galvanometer in the controlling mechanism marked *B* in Plate XCV. *G*, Multiple-point switch for connecting the resistance thermometers for the metal walls and air of the chamber with the Wheatstone bridge for measuring their temperatures. *H*, Tube conducting air from the respiration chamber to the rotary air pump. *I*, Tube conducting air from the purifying devices to the respiration chamber.


I
F
H
D
E
B
A


A
B
E
D

PLATE XCV

Devices for controlling and recording temperatures

A, Mechanism for shifting the contact on the rheostats controlling the current for heating the outer walls of the calorimeter chamber and the ingoing air. The rheostats are almost entirely hidden at the rear of the case inclosing the mechanism. *B*, Ratio coils for the four bridges governing the action of the shifting mechanism *A* are combined in this box, together with means for checking the constancy of the resistance of the coils and for correcting slight inequalities in them and also to compensate for small differences in the pair of resistance thermometers forming the other arms of each bridge. *C*, Mechanism for shifting the contact on the rheostat controlling the current in the heating coil in the final heater, shown at *E* in Plate XCIV. The rheostat is below the case inclosing the shifting mechanism. *D*, Temperature-difference recorder (self-balancing Wheatstone bridge) for continuously recording the difference between the temperature of the water entering and that leaving the heat absorber. *E*, "Check box" containing the ratio coils of the bridge for temperature difference measurements and coils for extending the range of differences measured, with means for checking the constancy of the resistances of the coils and the accuracy of the recorder readings and also for compensating for slight differences in the resistance of the thermometer coils when they are at the same temperature.

42721°—16——5

ADDITIONAL COPIES

OF THIS PUBLICATION MAY BE PROCURED FROM
THE SUPERINTENDENT OF DOCUMENTS
GOVERNMENT PRINTING OFFICE
WASHINGTON, D. C.
AT
25 CENTS PER COPY
SUBSCRIPTION PRICE, $3.00 PER YEAR

▽